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## CHLORPROPAMIDE AND DIABETES MELLITUS\*

*Consulting Editor*

MARTIN G. GOLDNER

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\* This series of papers is the result of a conference on *Chlorpropamide and Diabetes Mellitus* held and supported conjointly by The New York Academy of Sciences and Chas. Pfizer & Co., Inc., Brooklyn, N.Y., on September 25-26 and 27, 1958.

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## PREFACE

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A new era in the field of diabetes mellitus has been ushered in with the introduction of effective oral hypoglycemic agents particularly the sulfonylureas. The great impact made by these agents was evident in the success attending the conference on *Sulfonylureas and Related Compounds* sponsored by The New York Academy of Sciences in February 1957. It was apparent from this conference that compounds such as tolbutamide are to be added to the armamentarium useful in the management of diabetes. These substances appear capable of controlling the condition in adult onset diabetes with

only because of their use in the treatment of diabetic states but also because they served to rekindle interest and further research in the etiology of diabetes in the mechanisms of insulin synthesis storage and release and in the role of the liver and peripheral tissues in carbohydrate metabolism.

The present conference centers about sulfonylurea derivatives particularly chlorpropamide. It is becoming increasingly evident that incorporation of members of the halogen series into the structure of organic molecules often enhances their biological activities. This is true of hormones of adrenocortical origin in which for example substitution of halogens such as fluorine into the 9- and 6 alpha positions results in considerable augmentation of certain physiological actions. A similar situation is seen in the synthesis of thyronine derivatives in which iodine is superior to bromine and chlorine. This concept also extends to the sulfonylurea compounds notably chlorpropamide.

We look forward to further detailed information on topics relating to the mechanism of action of the sulfonylureas particularly their influence on insulin metabolism to a comparison of the relative effectiveness and toxicity of the newer derivatives especially chlorpropamide and to the extension of their use in the treatment of diabetic states in man. All of these topics are discussed in this monograph.

## INTRODUCTORY REMARKS

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It is my hope that the presentations and discussions comprising this monograph will bring us another step forward in the understanding of the role that the hypoglycemic sulfonylurea derivatives can play in the management of diabetes mellitus

When in 1942 the first chance observations were made at the University of Montpellier in southern France about the hypoglycemic action of a new sulfonamide, Loubatières foresaw that at last the long and so far rather fruitless search for an oral drug for the treatment of diabetes mellitus had reached a new phase and that a promising agent was finally at hand

However, it took another chance observation, this time by German investigators, and another decade until the first such drugs—tolbutamide and carbutamide—could be introduced into the practice of medicine. During the past few years nearly 1000 other sulfonylurea derivatives have been developed and evaluated in various laboratories. Chlorpropamide, or Diabinese, is one of these compounds. Because of such special characteristics of this preparation as its effectiveness in rather small doses and its lasting action, it was given an extended clinical tryout. The reports of these trials included in these pages will give us an opportunity to decide whether a new and practically useful agent has come into our hands in addition to those presently available

## Part I Pharmacological Studies

### GENERAL PHARMACODYNAMICS OF THE HYPOGLYCEMIC ARYLSULFONAMIDES

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The question of the general pharmacodynamics of the hypoglycemic arylsulfonamides of which the following\* is a brief survey has been the subject of a number of our more detailed articles<sup>1-10</sup>. We shall discuss here only the essential points in regard to this important question.

First we should record the fact that the basic experimental discoveries made in our laboratory between 1942 and the present time have been con-

#### 3-*p*-tolylsulfonylurea (D 860 tolbutamide)

The compound 1-propyl-3-(*p*-chlorobenzenesulfonyl)urea (P 607) chlorpropamide is still under study and at present some reservations must be expressed regarding it. Although its mechanism of action is probably the same as that of the compounds mentioned above this is not established also we are not well informed as to its toxicity and tolerance. It is the purpose of

sulfonamide or arylsulfonamide as seems suitable

#### *Mechanism of Action Experimental Study*

The fundamental and elemental role played by the endocrine pancreas (the beta cells of the islets of Langerhans) and insulin characterizes the mechanism of action of the arylsulfonamides.

As long ago as 1944 we demonstrated that the sulfonamides do not produce hypoglycemia in the totally depancreatized dog but that if even a fraction of the pancreas containing some islets of Langerhans is left in place the hypoglycemic action of these substances is manifested. When these substances are introduced into the organism they come into contact by way of the blood stream with the islets of Langerhans for which they have a special tropism they are pancreatotropic.

They act as stimulants to the beta cells from which they release a certain amount of endogenous insulin into the blood. Numerous experimental deductions support this statement.

\* This account is a revised version of that presented at the meeting of the Third International Congress of the International Diabetes Federation held from July 21 to 25 1958 at Düsseldorf Germany.

The effects of these compounds are as follows (1) the beta cells lose some of their intraprotoplasmic insulin granules, (2) the insulin content of the pancreatic tissue diminishes in response to the development of glycemia, (3) as shown by us in 1946 by means of experimental pancreatic jugular anastomosis, the hypoglycemic activity of the blood leaving the pancreas increases considerably a fact since confirmed in several ways, especially by measuring the insulin activity of the blood plasma leaving the pancreas

Recently we resumed these experiments and showed that the venous

before the action of the drug

the  
ate

sulfamides are hypoglycemic

It is also known that several days of sulfonamide administration produce in the normal animal a hyperplasia of the islet beta cell system and stimulate the formation of new beta cells that come into being at the expense of cells of the exocrine pancreatic gland (the centroacinar cells, cells of the excretory canaliculi the acinar cells) Here is a demonstration of the validity of Laguesse's theory that the cells of the exocrine gland may be the origin of endocrine cellular elements

In 1956 we studied the roles of the endocrine glands other than the pancreas in the mechanism of action of the arylsulfonamides The following experiments carried out in our laboratory demonstrate the fundamental role of the pancreas

Using the same dog, we removed, in several operative procedures, the following endocrine glands, one after another, in this order (1) the two adrenals (cortical and medullary tissues) (2) the thyroid, the four parathyroids, and the genital glands, and (3) the entire hypophysis

After each of the operative procedures the sulfonamide proved to be hypoglycemic, except following the removal of the entire pancreas However, it is clear that hypophysectomy and, especially, adrenalectomy sensitize the organism to the hypoglycemia induced by the sulfamides The sulfonamides retain their hypoglycemic action in the hepatectomized animal, provided the pancreas is intact However, this does not imply that the liver plays no role

The glycogenic action that these compounds exert upon the liver, demonstrated by us in 1944, is the basis for postulating that the sulfonamides act by inhibiting certain enzymes (glucose 6 phosphatase, reactivation of phosphorylase) However, these effects do not seem to be specific and are manifest only at high concentrations of the drugs Moreover, the inhibition they produce is only partial

Our recent experiments upon the dog subjected to a preliminary eight-day fast, which slows insulin secretion, show clearly that liver glycogen formation can be explained by the effects on the liver cell of endogenous insulin released by the sulfonamide Furthermore, we observed that administration of

sulfonamide also induces an accumulation of glycogen in muscle, that is to say it affects the peripheral tissues. Both of these results, the hepatic and the muscular, of the sulfonamides may be reproduced completely by

depancreatized dog is preserved completely. These facts show that the sulfonamides do not seem to inhibit profoundly the enzymatic reactions involved in glycogenolysis or hepatic glycogen formation in the physiological conditions under which we operated.

Furthermore, we showed at the same time as Houssay and his collaborators that the sulfonamides in massive doses potentiate the hypoglycemic effects of insulin even after ablation of the pancreas. It has not been demonstrated that this potentiation of the effects of insulin is due to an inhibition of insulinase. We have other hypotheses. Whatever the explanation, the release of endogenous insulin precedes the subsequent intensification of the effects of this hormone. A possible action of the sulfonamides on the peripheral tissues in the absence of insulin or the pancreas has not been demonstrated. Recent study of the peripheral arteriovenous differences in blood sugar after sulfonamide and after insulin shows striking similarities.

On the other hand, we have shown that the administration of sulfonamide is capable of accumulating glycogen in the striated muscle of a dog subjected to prolonged preliminary fasting. We have reason to believe that endogenous insulin released by the sulfonamide and not wholly arrested by the hepatic barrier is responsible for these peripheral effects.

In addition to their hypoglycemic action, the sulfonamides possess an antidiabetic action that is exhibited under certain experimental conditions in the dog with alloxan diabetes. We have shown that, if the diabetes is severe (that is to say, if all of the beta cells have been destroyed by the alloxan) the sulfonamides are inactive, they may even hasten the evolution of the diabetes toward acidosis.

On the other hand, if the diabetes is of moderate intensity, the action of the sulfonamides is evident. The different manifestations of the diabetes progressively diminish and then disappear. After several weeks the treatment can be discontinued, and the animal is "cured." New beta cells have developed in the pancreas of these animals. We have shown that these cells are functionally normal, since they are perfectly capable of regulating the blood sugar, they are therefore sensitive to the delicate stimuli for insulin secretion. They can be stimulated by the sulfonamides and, in turn, they can be destroyed by alloxan.

This new islet apparatus whose functional activity exists thanks to the

gressively hyperglycemic by the end of six months. This shows the impor

tance of a controlled diet for maintaining the benefits obtained from the sulfonamides

### *Mechanism of Action in Man*

Can proof of hypoglycemic antidiabetic action be established in normal or diabetic man? The hypoglycemic action of the sulfonamides is admitted without reservation in the case of normal man. On rare occasions these substances cause serious hypoglycemic accidents that, in any case, are reversible by glucose. In a man totally without pancreatic tissue these substances are inactive, they maintain their activity if a small amount of endocrine pancreas remains in the organism.

sugar. The effects observed depend, therefore, on the respective and opposite roles played in establishing the total diabetic state by the pancreatic component, on the one hand, and the extrapancreatic diabetogenic component on the other. This explains the variable results observed following the use of sulfonamides when glandular hyperfunction (anterior pituitary or adrenal cortical) is beginning, or when this hyperfunction is being reduced. The hemosiderin depot observed in the beta cells of the islets of Langerhans probably is not unrelated to the lack of action of the sulfonamides in hemochromatosis (bronze diabetes). This behavior confirms once more the tropism of the sulfonamides for the beta cells of the pancreas.

In cirrhosis of the liver with diabetes the activity of the sulfonamides is variable and depends on many factors. In the diabetic man under insulin

sulfonamide added its effects to that of the injected insulin? Do other mechanisms enter in? These questions are still unanswered.

We have recently answered several questions in regard to sulfamide treatment. It has been stated, for example, that the sulfonamides are not physiological agents. That is true, but their pharmacological action causes endoge

explained by the degree of lesion of the beta islet cells of the pancreas at the different stages that represent the various degrees of intensity of the diabetes.

The classification of diabetic subjects in terms of their different responses to the sulfamides is a strong argument in favor of our theory of the mechanism of action of these substances. Young diabetics, refractory to the sulfamides,

have severe beta cell lesions in their pancreas, the insulin content of this organ is low, the stimulation of its cells by glucose administration releases practically no detectable insulin into the blood.

On the other hand, diabetics stricken with the disease at a mature age have numerous intact beta cells in their pancreas, the insulin content of this

acknowledged that the sulfonamides seem to act especially in subjects exhibiting a certain degree of sluggishness or insufficiency of the insulin secretory processes. Despite the considerable amount of work accomplished since 1946, the theory that emerges remains in conformity with the one we have maintained since then and will continue to maintain, because it is well founded.

There is a question as to whether the chronic stimulation of the beta cells may not finally bring on their exhaustion and an aggravation of the diabetes. Taking into consideration our long experience, it does not seem that this contingency often arises when the sulfonamides are correctly used in subjects who are really suitable. When this condition arises, it is necessary to take into account deviations from the diet, the way in which the sulfonamides are used, and the spontaneous aggravative evolution of certain cases of diabetes before the sulfamides themselves can be held entirely responsible. Conditions called refractory to the sulfonamides may appear, they may be explained in part by the phenomenon of tachyphylaxis.

#### *Hypoglycemic Sulfamides and the Prospects for Extended Remission of Diabetes Mellitus*

In conclusion we should like to explain why, in our opinion, the hypoglycemic and antidiabetic sulfamides offer favorable prospects for prophylaxis or extended remission of diabetes.

Accumulated clinical data show two types of results in certain elderly diabetics or even in young diabetics (provided their diabetes is recent and not severe) a sulfamide treatment carried on for several weeks can cause the diabetes to disappear. In many cases the benefits last for several months after suspension of sulfamide treatment. The sulfamides have, therefore, an antidiabetic action in man as well as in animals.

It is possible that the extended remission of diabetes, which is not a final cure, is explained by at least partial regeneration of the endocrine pancreatic system. If this is true why not attempt to use the sulfonamides as prophylactic and retarding agents in subjects predisposed to this disease?

There is another point that should engage our attention, since it presents a problem and indicates a line of research. It involves the observation of

the 'cured' animal the blood sugar is normal and, if the diet is correctly controlled, the 'cure' lasts. Lesions caused by alloxan were pancreatic,



the sulfonamides have compensated for the pancreatic lesions at least in part by facilitating the regeneration of the beta cells, glycemie equilibrium has returned and will be prolonged if certain experimental conditions are respected. In extended remission of diabetes in man, blood sugar is almost never normal but is definitely above 100 to 120 gm of glucose per liter. After several weeks or months the diabetes progressively resumes with all its former intensity. What has happened?

The sulfamide treatment it is true has partially restored the endocrine

of diabetes

Thanks to the research accomplished in the domain of the hypoglycemic sulfonamides an important advance has been made, but our task as seekers is not yet over. Now we must undertake to detect and neutralize the harmful extrapancreatic influences. It is probable that we shall not be too long without weapons against these factors. We should succeed in this because these influences often are exerted only temporarily in the course of a lifetime (period of somatic growth, premenopausal period), and science should not lose the hope of overcoming them once they are detected.

The "cure" of experimental diabetes is possible if the disease has not exceeded a certain degree of severity. It is not unreasonable to believe that we are on the way to realizing within organically permitted limits at least, the extended remission of human diabetes mellitus. If we arrive at this result the challenge offered by the hypoglycemic and antidiabetic sulfonamides sixteen years ago to diabetologists will not, from the scientific, the social and the human points of view have been in vain.

### References\*

- 1 LOUBATIÈRES A 1946 Physiologie et pharmacodynamie de certains dérivés sulfamidés hypoglycémisants. Contribution à l'étude des substances synthétiques à tropisme endocrinien. 93 pp. Thèse Doct. Sci. Nat. No 1001. Causse Graille et Castelnau. Montpellier France.
- 2 LOUBATIÈRES A 1945 Presse méd 63 1701 1728
- 3 LOUBATIÈRES A 1956 Ann N Y Acad Sci 67(7) 185
- 4 LOUBATIÈRES A 1957 Ann N Y Acad Sci 71(1) 4 192
- 5 LOUBATIÈRES A 1957 Ann endocrinol Paris 18 161 260
- 6 LOUBATIÈRES A 1957 Diabetes J Am Diabetes Assoc 6 408
- 7 LOUBATIÈRES A 1957 Antibiotica et Chemotherapia Progr 4 69 114 Karger Basel Switzerland
- 8 LOUBATIÈRES A 1957 Rapport II<sup>e</sup> Réunion Endocrinol Langue Française Paris 30, 332 Masson Paris France
- 9 LOUBATIÈRES A 1957 Bull soc chim biol Suppl 4 63 75
- 10 LOUBATIÈRES A 1958 Presse méd 66 1175 1229

\* A detailed bibliography on the subject of hypoglycemic arylsulfonamides will be found in the author's published works.

# STUDIES ON THE EFFECT OF ORAL ANTIDIABETIC COMPOUNDS ON GLUCOSE TOLERANCE, ON ISLET CELL STRUCTURE, AND ON THE *IN VITRO* METABOLISM OF ISOLATED MUSCLE

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The mode of action and the locus or loci of action of the individual oral antidiabetic compounds have not been fully elucidated. We shall present briefly the results of three kinds of studies in which we have tried to obtain some information about the action of these compounds.

## *Oral and Intravenous Glucose Tolerance During the Action of Oral Antidiabetic Compounds*

The first study is of glucose tolerance in diabetic patients.<sup>1</sup> FIGURE 1 shows the average oral glucose tolerance curve before and during the administration of carbutamide or tolbutamide in 18 patients. The antidiabetic effect is

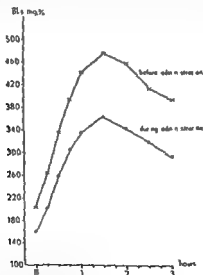


FIGURE 1. Average curves showing oral glucose tolerance before and during the administration of carbutamide or tolbutamide.

quite obvious. It is not only a parallel displacement of the curve that occurs, the area of increase, that is, the area above fasting level, is significantly decreased during the action of the sulfonylureas. FIGURE 2 shows the results of intravenous glucose tolerance tests. The individual  $k$  values,

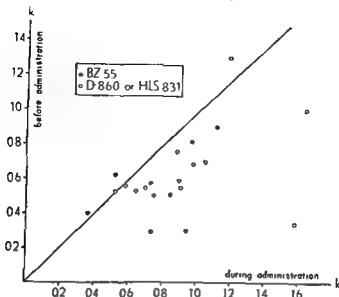


FIGURE 2  $k$  Values for intravenous glucose tolerance curves before and during the administration of carbutamide (BZ 55) or tolbutamide (D 860 or HLS 831)

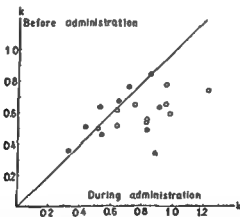


FIGURE 3  $k$  Values for intravenous glucose tolerance curves before and during the administration of chlorpropamide. Symbols  $\bullet$  = 1.75 gm during a period of 48 hours  
 $\circ$  = 3 to 5 gm during a period of 48 to 96 hours

expressing the slope of the decrease in blood sugar in each of the patients before and during treatment with sulfonylureas, are plotted against each other. An antidiabetic effect on the intravenous glucose tolerance is seen, the points clearly tending to accumulate below the 45° identity line.

sugar<sup>2</sup> Even by these devious ways we find that an antidiabetic effect although less significant, can be shown to occur in the intravenous glucose tolerance test Baird and Duncan<sup>3</sup> have published findings very similar to ours but, by using the blood sugar excess values they failed to demonstrate any significant effect

TABLE 1 shows the serum inorganic phosphate during glucose tolerance tests before and during the action of sulfonylureas It appears that the

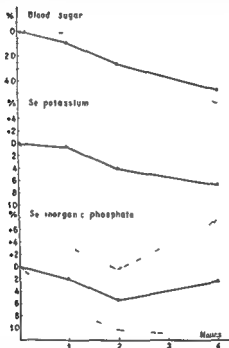


FIGURE 4 Blood sugar serum potassium and serum inorganic phosphate 1 2 and 4 hours after oral administration of 1 gm chlorpropamide Values are shown as per cent changes from the before values Dotted lines represent standard deviation

amelioration of the glucose tolerance was not accompanied by any change in the phosphate response

FIGURE 3 shows the results of intravenous glucose tolerance tests in 20 diabetic patients before and during administration of chlorpropamide It appears that 1 gm /day of this compound produces an amelioration of the glucose tolerance similar to the one found with carbutamide or tolbutamide However chlorpropamide increased to some extent the response of the inorganic serum phosphate to glucose (TABLE 1) and when given in the fasting state it produced a small but significant fall of serum phosphate and of serum potassium (FIGURE 4)

TABLE 1

AVERAGE SERUM INORGANIC PHOSPHATE DURING ORAL AND INTRAVENOUS GLUCOSE TOLERANCE TEST BEFORE AND DURING THE ADMINISTRATION OF CARBUTAMIDE (C), TOLBUTAMIDE (T) OR CHLORPROPANIDE (CP)

Glucose tolerance test	Antidiabetic compound	No of patients	Time after oral or I V glucose (min)				Max fall	Percentage fall
			0	60	120	180		
Oral	0	18	3.8	3.6	3.5	3.4	0.41	10.8
Oral	C or T	18	3.7	3.4	3.3	3.4	0.41	11.1
Intravenous	0	21	3.7	3.5				5.4
Intravenous	C or T	21	3.6	3.4				5.6
Intravenous	0	20	3.6	3.5				3.8
Intravenous	CP	20	3.8	3.5				7.1

It is not clear if this difference between the response of the inorganic phosphate to carbutamide and tolbutamide, on the one hand, and to chlorpropamide, on the other, is due to a difference in the mode of action of these compounds. It should be noted that under certain circumstances a fall of serum inorganic phosphate has been reported to occur after administration of tolbutamide.<sup>4, 5</sup>

#### *Pancreatic Islets of the Rat During the Administration of Sulfonylureas*

The second type of study concerns the histological structure of the pancreatic islets in the rat during the administration of sulfonylureas.<sup>6</sup>

Normal female rats were injected intraperitoneally with *p* aminobenzene-sulfonamidoisopropylthiodiazole (IPTD), carbutamide, or tolbutamide in

experi

The islet

following

the details of the procedure described by Gomori.<sup>7</sup> The results are shown in TABLE 2. No difference in the structure or granulation of the alpha or beta cells of the controls or of the injected animals was obtained. Synthalin A

tion in the rat. They differ in the same respect from the results of studies in the calf<sup>9</sup> and in the rabbit.<sup>10</sup> This has been interpreted as histological support for the hypothesis that the oral antidiabetic compounds act by increasing the production and/or output of insulin from the pancreatic islets. In our laboratory, working with rats, we have not been able to produce any histological evidence for this hypothesis.

However we emphasize that we have had considerable difficulty with the Gomori stainings, which seem to vary somewhat from day to day, giving

TABLE 2  
HISTOLOGICAL CHANGES IN PANCREATIC ALPHA AND BETA CELLS OF RATS AFTER  
ADMINISTRATION OF VARIOUS ANTIDIABETIC DRUGS

Drug	No of animals	No of controls	Total dose (mg)	Histological changes of	
				Alpha cells	Beta cells
Carbutamide	75	29	75-315	■	■
Tolbutamide	44	11	75-3375	0	0
IPTD	28	9	75-3375	0	0
Synthalin A	10	3	20	+++	0
Total	157	52	—	—	—

the illusion of a change in the granulation of the beta cells. We have found that it was always necessary to carry the control specimens in the same run all the way through the entire procedure.

#### *In vitro Studies of Isolated Rat Diaphragm*

The third type of experiment deals with the action of sulfonylureas on the isolated diaphragm of the fasting rat.<sup>11</sup>

For these experiments the usual Gemmil technique was used. The medium was Gey and Gey's buffer with 300 mg per cent of glucose. The antidiabetic compounds were either injected into the animals a few hours before the experiment or were added to the medium. Each experiment included 6 to 12 treated and 6 to 12 control hemidiaphragms. In injection experiments we attempted to produce a blood level of about 10 to 50 mg per cent of carbutamide. In additional experiments the medium contained from 6 to 100 mg per cent of the antidiabetic compound.

In injection experiments with carbutamide and in experiments in which carbutamide, tolbutamide, IPTD, or phenethylidiguamide was added to the medium, a significant rise in the glucose uptake was observed consistently. No change occurred in the glycogen content of the muscle except with phenethylidiguamide, which produced a fall in the muscle glycogen.

The results of the experiments with antidiabetic compounds added to the medium are summarized in figures 5 and 6.

Our findings demonstrate that IPTD, carbutamide, and tolbutamide can increase the uptake of glucose in the isolated muscle tissue without affecting glycogen deposition; that is, changes in the metabolism of the muscle that differ from the action of insulin on isolated muscle tissue. Similar results have been obtained by Canal *et al*<sup>12</sup> and by Pletscher and Gey,<sup>13</sup> but other investigators have reported negative results of such studies.<sup>14-17</sup> Our results, using phenethylidiguamide, are in accordance with the results reported by Williams *et al*.<sup>18</sup>

The results obtained in our laboratory indicate that the action of the

antidiabetic compounds on the muscle is independent of the presence of active beta cells that it is not brought about by an increase in the production and delivery of insulin from the pancreas

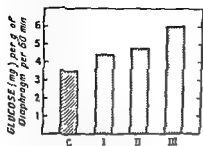


FIGURE 5 Average uptake of glucose

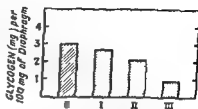


FIGURE 6 Average glycogen content of the isolated rat diaphragm after incubation in glucose-containing medium with various oral antidiabetic compounds C, I, II, III and concentration are as in FIGURE 5 III is significantly lower than C, I, and II

I carbutamide, II tolbutamide, III phenethylbiguanide concentration 35 mg per cent. The difference between each of these average values is statistically significant

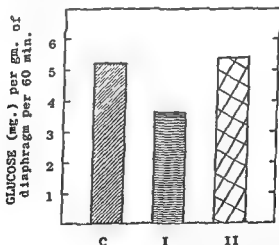


FIGURE 7 Average uptake of glucose by the isolated diaphragm of alloxan diabetic rats C, controls I alloxan diabetic rats, II alloxan-diabetic rats (concentration of carbutamide in the medium is 35 mg per cent) C and II are significantly higher than I

We have studied also the effect of carbutamide on the glucose uptake of diaphragms isolated from alloxanized rats. The rats were moderately to severely diabetic (blood sugar 200-300 mg per 100 ml) and had a glycogen content (FIGURE 8)

the same glycogen

Of course these results are in disagreement with the hypothesis that the action of the oral antidiabetic compounds require the presence of beta cells capable of increasing the output of insulin. However it is not possible to prove that an alloxanized animal is an insulin free organism and we are well aware of the fact that our results with alloxanized rats do not disprove the hypothesis supported by many other kinds of evidence that the action of these compounds requires the presence of insulin. We do believe that it is important to distinguish the hypothesis of action by increased insulin secretion from the hypothesis of insulin as a permissive agent for the action of the oral antidiabetic compounds.

There is good evidence from several sources<sup>10-12</sup> that the oral antidiabetic compounds reduce the output of glucose from the liver but it is known that they are active even in the absence of this organ<sup>13-15</sup>. The results presented here suggest that an action on the peripheral uptake of glucose might also contribute to the fall of blood sugar observed after administration of these compounds.

### Discussion

We did not measure oxygen uptake in these experiments but I do not think that lack of oxygen can explain the increase of glucose uptake observed in our experiments with oral antidiabetic compounds. We are working with a bicarbonate phosphate buffer and an atmosphere of carbogen. In our control experiments with nonhypoglycemic sulfonylureas we did not find any increase in glucose uptake. Our system is highly sensitive to insulin both on glucose uptake and on glycogen deposition.

There is some evidence of a more general enzyme inhibition with very high concentrations of sulfonylureas. A lack of increase of glucose uptake by the isolated diaphragm with such high concentrations has also been reported from other laboratories.

### Summary

In clinical studies of the oral and intravenous glucose tolerance an increase of glucose tolerance was observed during the administration of carbutamide, tolbutamide or chlorpropamide to diabetic patients. Serum inorganic phosphate was unchanged during the action of carbutamide and tolbutamide. With chlorpropamide a slight decrease in serum inorganic phosphate and in serum potassium occurred.

Histological studies of the pancreatic islets of the rat showed no changes in the alpha or beta cells during the administration of low or high doses of IPTD, carbutamide or tolbutamide for short or long periods of time.

*In vitro* studies of the isolated rat diaphragm showed a rise in the glucose uptake when carbutamide was injected previously; a similar rise was observed on the addition of IPTD, carbutamide or tolbutamide to the medium *in vitro*. No change in the glycogen content of the diaphragm was observed. The same results were obtained using diaphragms from alloxanized rats with moderate or severe diabetes. Phenethylidiamide caused a rise in



the glucose uptake and a fall in the glycogen content of the isolated rat diaphragm

### References

- 1 ESKJAER JENSEN S K LUNDBAEG B MØLLER & O J RAFAELSEN 1958 The action of oral antidiabetic compounds on the blood sugar and serum inorganic phosphate after oral or intravenous administration of glucose Acta med scand 160 67
- 2 AMATUZIO D S F L STILIZMAN M J VANDERBILT & H S NESBITT 1953 Interpretation of the rapid intravenous glucose tolerance test in normal individuals and in mild diabetes mellitus J Clin Invest 32 423
- 3 BAIRD J M & L J P DUNCAN 1957 The interpretation of the intravenous glucose tolerance test Clin Sci 16 147
- 4 GOETZ F G A S GILBERTSEN & V JOSEPHSON 1956 Acute effects of Orinase on peripheral glucose utilization Metabolism 5 788
- 5 FRANKLEY T I, B SPAL M M CAMUS & J FOLEY 1957 A concept of the mechanism of sulfonylurea induced hypoglycemia based on studies of glucose and pentose disposition in man Ann N Y Acad Sci 71(1) 81
- 6
- 7
- 8 KRACHT J & J C RAUSCH STROOMANN 1956 Das Inselzellsystem unter  $N_2$  auf family  $N_2$  n butylcarbamid Naturwiss 43 180
- 9 BANDER A A HALSSLER & J SCHOLZ 1957 Ergänzende pharmakologische Untersuchungen über Rastinon Deut med Wochschr 82 1557
- 10 LA
- 11 R
- 12 CA  
derivati ureici ad azione ipoglicemizzante Boll soc ital diol diet 34 471
- 13 PLETSCHER A & K F GEY 1957 Über die Wirkung blutzuckersenkender Sul  
half Element 13 447
- 14 Some in vitro
- 15 of substituted
- 16 disposition of  
Ann N Y
- 17 tolbutamide  
hypoglycemia in animal and human subjects Ann N Y Acad Sci 71(1) 62
- 18 WILLIAMS R H J M TYBERGHEIM P M HYDE & R L NIELSEN 1957 Studies  
pharmoth 11 guanide Metabolism 6 311  
amide on the isolated diaphragm
- C M TERMINE 1957 Obser  
34
- 19 1956 Hypoglycemic action  
Effect on output of glucose by the liver Proc Soc Exptl Biol Med  
92 340
- 22 TARDING F & P SCHAMBYE 1958 The action of sulfonylureas and insulin on the glucose output from the liver of normal dogs Z Endokrinol 36 222
- 23 DULIN W E & R L JOHNSON 1957 Studies concerning the role of the liver in the hypoglycemic response of animals to tolbutamide Ann N Y Acad Sci 71(1) 177

## THE PHARMACOLOGY OF CHLORPROPAMIDE

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Ever since it was recognized that insulin could not be used orally in the treatment of diabetes mellitus both synthetic compounds and natural products have been investigated for hypoglycemic activity pharmacologically and clinically. Severe side effects and/or insufficient activity made many of the previous attempts futile or impractical.

The recent extensive studies of certain sulfonylureas as orally active hypoglycemic agents<sup>1, 2</sup> are the result of a concentrated research effort of the last sixteen years.<sup>3</sup> When the therapeutic value of the sulfonylureas was recognized the search for the most effective compounds of the series was intensified. Chlorpropamide was selected as a result of an extensive screening program. Its high degree of hypoglycemic activity and prolonged duration of action as evidenced by animal experimentation warranted thorough pharmacological and clinical studies.<sup>4</sup>

This paper summarizes the present status of pharmacological investigation with chlorpropamide taking into account previous reports by Salgado *et al.*<sup>5</sup> and Root.<sup>6</sup> Emphasis is placed on new aspects; data corroborating earlier findings are referred to briefly.

### Materials and Methods

The results reported were established in mice (Webster strain) rats (Wistar strain) cats dogs and rhesus monkeys. Routine pharmacological and biochemical methods were employed unless otherwise indicated.

All blood sugar determinations were performed according to the method of Folin and Malmros.<sup>7</sup> The animals were fasted for 18 hours previous to the withdrawal of blood samples. Liver and muscle glycogen were determined by the method of Seifter.<sup>8</sup> Chlorpropamide blood levels were evaluated by a spectrophotometric method developed by Toolan and Wagner.<sup>9</sup>

Chlorpropamide was administered orally in most experiments. Where parenteral administration was employed the sodium salt of chlorpropamide was used (solubility in water > 50 per cent pH of 1 per cent solution = 8.5). Radioactive material consisted of S<sup>35</sup> labeled chlorpropamide.

### Results

*Hypoglycemic activity of chlorpropamide.* As reported previously,<sup>5, 6</sup> chlorpropamide was found to cause a prolonged hypoglycemic effect in both rats and dogs. Single oral doses of 20 mg/kg or higher were required to obtain a definite effect. Calculated on a mg/kg basis, this is at least twice the recommended average human dose (7 to 10 mg/kg/day). When compared to tolbutamide in these two species chlorpropamide was somewhat slower in its onset but more prolonged in its duration of action, the maximal hypoglycemic effect of both compounds was of approximately equal magnitude.

Root<sup>6</sup> had observed that, using both duration of action and degree of hypoglycemia as criteria for evaluation, in both rats and dogs chlorpropamide was more active on a weight basis than tolbutamide. The hypoglycemic effects of chlorpropamide and tolbutamide were also compared in rhesus monkeys following single oral administration. The results of such an experiment are shown in FIGURE 1. At dose levels of 10 mg/kg orally a definite hypoglycemic response was obtained that was slower in onset but considerably greater in magnitude and more prolonged in its duration of

#### HYPOGLYCEMIC ACTIVITY OF CHLORPROPAMIDE IN THE MONKEY

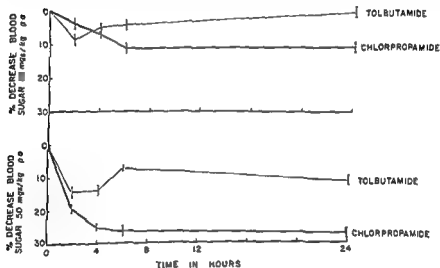


FIGURE 1. Top tracing crossover studies in 4 monkeys illustrating the hypoglycemic effect of chlorpropamide as compared to tolbutamide.

action than with tolbutamide. At doses of 5 mg/kg orally (not shown on FIGURE 1) a definite blood sugar lowering effect was observed with chlorpropamide whereas tolbutamide caused a slight and rather transient rise of blood sugar levels at that dose. At 50 mg/kg, which is an excessive dose, both compounds caused pronounced hypoglycemia, chlorpropamide again being more active and more prolonged in its effect than tolbutamide. Therefore in the rhesus monkey chlorpropamide was active in dose levels that compare well to those used in the human being.<sup>10</sup> The results also indicate that rhesus monkeys, much like human beings, are more susceptible to chlorpropamide than either dogs or rats.

In view of the long duration of action of chlorpropamide, the question arose whether chlorpropamide had cumulative effects. To clarify this point, groups of four dogs were given doses of 5, 25, and 50 mg/kg of chlorpropamide orally for several weeks. Blood sugar and chlorpropamide blood levels were determined at regular intervals. The results of this study are shown in FIGURE 2. It can be concluded that continued administration of subthreshold doses of chlorpropamide did not result in a hypoglycemic effect, even after 2 weeks of daily medication. However larger doses caused a gradual decrease of the blood sugar that was paralleled by a rise in serum

### CHRONIC ADMINISTRATION OF CHLORPROPAMIDE TO DOGS

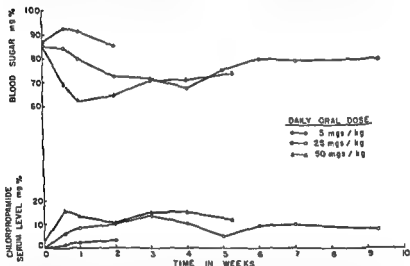


FIGURE 2 Comparison of hypoglycemic activity and blood levels of chlorpropamide in groups of 4 dogs following daily oral administration at various dose levels. Each point represents an average of 4 values. All blood sugar determinations and chlorpropamide blood levels were made 24 hours following the last dose, the animals were in a fasting state.

concentration of chlorpropamide. After several days the blood levels of chlorpropamide stabilized and did not increase beyond a certain point. As noted by Root,<sup>6</sup> hypoglycemic effect and drug concentration in serum were not always in good correlation. Reduction or cessation of medication resulted in gradual reduction of chlorpropamide blood levels and return of blood sugar to normal values.

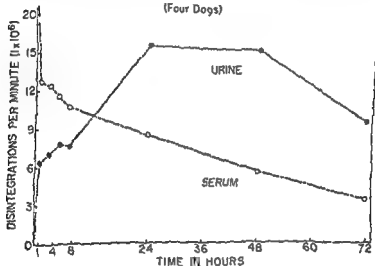
Since the method of Toolan and Wagner,<sup>8</sup> is not applicable for determination of chlorpropamide in urine, excretion studies were performed in dogs with radioactive  $S^{35}$  labeled chlorpropamide given intravenously. The results available are summarized in FIGURE 3. According to these data the radioactive material is excreted very slowly, only about 45 to 50 per cent of the material given being accounted for after 72 hours. Chromatographic

studies are in progress to determine whether chlorpropamide is excreted unchanged in the dog or whether it is metabolized

**Toxicology** In acute oral, intravenous, and intraperitoneal toxicity studies in mice, rats, and dogs, chlorpropamide was slightly more toxic than tolbutamide on a weight basis (TABLE 1), the species used, the age, and general status of the animal were important factors for survival following single doses. This finding is in accord with the more potent hypoglycemic activity of chlorpropamide over tolbutamide

#### AVERAGE CANINE RADIOACTIVE CHLORPROPAMIDE, IV

SERUM AND URINE VALUES  
(Four Dogs)



Chronic toxicity experiments were performed in dogs, rats, and monkeys at various dose levels for periods ranging from 6 to 20 months. Dogs tolerated daily oral doses of 150 mg/kg, which corresponds to more than 10 times the recommended clinical dose in the human being. Except for

administration. Toxic symptoms observed were ataxia, tremors, muscular weakness, coma, and death.

Rats survived daily doses up to 500 mg/kg in their diet for 6 months to 1 year. A most interesting phenomenon developed in this species only at the high dose levels (FIGURE 4). Chlorpropamide caused a retardation in

TABLE 1  
ACUTE TOXICITY OF CHLORPROPAMIDE AND TOLBUTAMIDE

Drug	Species	Route	LD <sub>50</sub>	95% Confidence limits* mg/kg
Chlorpropamide	Mice	Oral	1675	1444-1943
Chlorpropamide	Mice	S C	780	710-856
Chlorpropamide	Mice	I P	760	693-834
Chlorpropamide	Mice	I V	500	431-580
Chlorpropamide	Rats	Oral	2390	1943-2940
Chlorpropamide	Rats	S C	760	603-942
Chlorpropamide	Rats	I P	575	454-724
Chlorpropamide	Rats	I V	590	486-714
Chlorpropamide	Dogs	Oral	approx 800	
Chlorpropamide	Dogs	I V	approx 575	
Tolbutamide	Mice	Oral	1830	1591-2105
Tolbutamide	Mice	I V	700	669-731
Tolbutamide	Rats	Oral	2490	2029-2936
Tolbutamide	Rats	I V	775	731-817

\* Whenever possible LD<sub>50</sub> and 95 per cent confidence limits were calculated according to the method of Litchfield and Wilcoxon. CMC (carboxymethylcellulose) was employed as a suspending agent. Rats and mice used were weanlings.

#### THE EFFECT OF CHLORPROPAMIDE ON THE GROWTH RATE AND FOOD CONSUMPTION OF MALE ALBINO RATS (SERIES II)

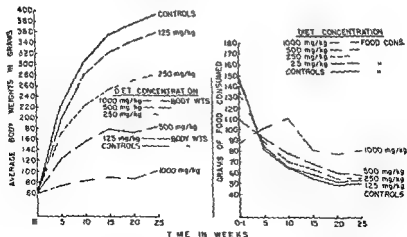


FIGURE 4. Growth-depressant effects of chlorpropamide in rats as evidenced by plotting average body weights of groups of twenty animals versus time (left graph). A dose response effect could be established clearly. Feed consumption (gm/kg) in the corresponding groups (right graph) indicates adequate food intake.

growth that was dose dependent the food intake of these rats however did not decrease proportionally. In order to check whether chlorpropamide interfered with the availability of certain amino acids essential for growth cysteine and/or glycine were added to the diet in equimolar concentration to chlorpropamide. Although glycine but not cysteine alleviated growth depression slightly these dietary adjustments failed to overcome chlorpropamide induced growth retardation.

Rhesus monkeys are presently being maintained on chlorpropamide orally in doses up to 200 mg/kg (about 30 times the blood sugar lowering dose) intermittent diarrhea in the high dose range is the only toxic symptom observable.

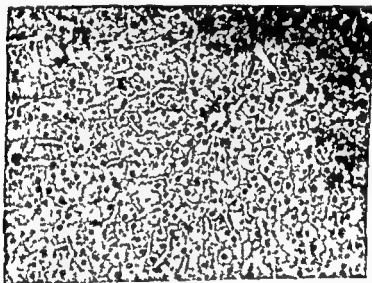


Fig. 6. Liver tissue from a dog treated orally. No signs of

Autopsies were performed on all animals sacrificed after periods of up to

As in all other

As in all other

As in all other

As in all other

As in all other

As in all other

As in all other

As in all other

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As in all other

As in all other

As in all other

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accord with the unimpaired liver function of the dog as evidenced by periodical liver function tests such as for alkaline phosphatase, thymol turbidity or Bromsulphalein (BSP).

*Mode of action.* Despite thorough pharmacological investigation qualitative differences between the mode of action of chlorpropamide and tolbutamide could not be established. Chlorpropamide showed a marked effect on glycogen metabolism as evidenced by increased glycogen content of the liver (FIGURE 6) on the other hand glycogen content of striated muscle was

unaltered. Insulin sensitivity was not increased by chlorpropamide (FIGURE 7), while glucose tolerance remained within the limits of normal. In our laboratories similar results were observed with tolbutamide. In accord with

several days (FIGURE 8). The phenomenon was reversible after discontinuation of medication for 72 hours. Furthermore alloxan diabetic rats showed no hypoglycemic reaction to chlorpropamide, whereas severe hypoglycemia

EFFECT OF CHLORPROPAMIDE 100 mgs/kg  
p.o. ON LIVER AND MUSCLE GLYCOGEN  
2 HOURS AFTER TREATMENT

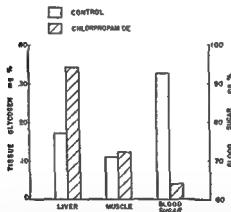


FIGURE 6. Glycogen contents in liver and muscle before and following chlorpropamide treatment in rats. Six rats were used for each determination at a dose level of 100 mg/kg orally. The animals were sacrificed 2 hours after treatment; the blood sugar determinations taken simultaneously indicate the effectiveness of the medication. The animals were fasted throughout the test. Chlorpropamide caused an increase in liver glycogen over the control but caused no change in muscle glycogen. This is in accord with data in the literature obtained with other sulfonylureas.

and death followed drug treatment of adrenalectomized rats (FIGURE 9). Similar experimental data are reported for tolbutamide.<sup>1,2</sup>

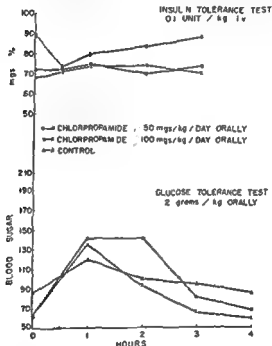
Chlorpropamide was also investigated thoroughly for pharmacodynamic actions other than those related to its hypoglycemic activity. It lacked specific blood pressure, respiratory, or diuretic effects in doses that caused profound hypoglycemia. There was no antagonism to histamine or acetylcholine in the isolated guinea pig ileum.

The most obvious symptoms following high doses of chlorpropamide in mice, rats, cats, and dogs are general weakness, associated with ataxia, and incoordination. They may occur within a few minutes after intravenous



administration of high doses to an unanesthetized animal. Patients receiving an overdose of chlorpropamide are known to complain of muscular weakness.<sup>10</sup> Hypoglycemia may be a factor involved, but glucose infusions were sometimes not adequate to alleviate these symptoms. On the other hand the possibility of a direct pharmacological effect of chlorpropamide on electro-

INSULIN AND GLUCOSE TOLERANCE TESTS  
IN DOGS AFTER A YEAR OF DAILY  
TREATMENT WITH CHLORPROPAMIDE



the response was 5.4 after four hours

lyte balance, muscular function, spinal reflexes, or central nervous system must be considered.

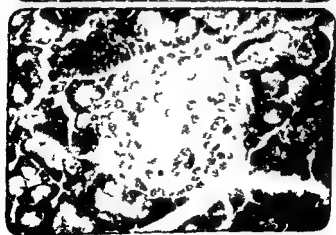
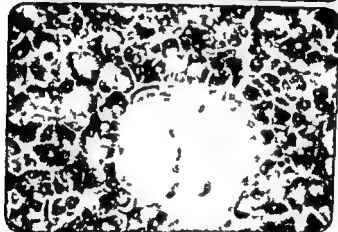
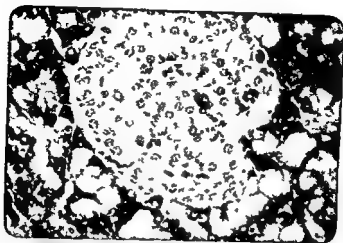
The electrolyte balance was studied in dogs who received repeated sublethal doses of chlorpropamide until a state of prostration was obtained. Sodium and potassium values in serum were within limits of normal with few exceptions (TABLE 2). On the other hand, it is noteworthy that these dogs appeared to respond more rapidly and more completely to combined infusions of glucose and isotonic saline solutions than to glucose alone.

TABLE 2  
 SERUM Na<sup>+</sup> SERUM K<sup>+</sup> (mEq/l) VALUES OF FEMALE MICE  
 ORAL DOSES OF CHLORPROPAMIDE  
 (Sample Taken 2 Hours Post Dose Unless Indicated)

Drug No.	Daily dosage chlorpropamide mg/kg	Prechlorpropamide		Post Rx† Shown		Post Rx Shown		Post Rx	
		Na	K <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Na	K <sup>+</sup>
J139	150 6X			Rx 5		Rx 13		Rx 21	
	200 16X	156 3	5 63	146 2	5 28	147 5	5 30	142 4	1
	300 27X								
J141	150 6X	not read	6 90*	Rx 5		Rx 13		Rx 21	
	200 16X			152 0	5 83	144 1	5 20	145 9	
	300 21X								
J163	150 6X			Rx 5		Rx 13		Rx 21	
	200 16X	147 2	5 34	150 0	5 74	145 1	5 25	140 8	4 4
	300 12X								
J162	150 6X			Rx 5		Rx 13		Rx 21	
	200 16X	161 4	7 78*	156 2	5 64	139 1	6 67	143 9	1
	300 16X								
J193	150 6X			Rx 5		Rx 13		Rx 21	
	200 16X	151 3	5 09	135 4	5 54	153 7	5 15	134 2	6 52
	300 5X								
	400 1X								
J165	150 3X			Rx 4		Rx 12		Rx 19	
	200 10X	154 9	5 71	145 1	5 00	140 8	5 19	146 5	2 94
	300 13X							150 0	4 0
J203	150 6X			Rx 5		Rx 13		Rx 21	
	200 16X	146 7	5 39	153 4	5 25	148 5	5 00	136 7	4 59
	300 1X							144 8	3 66
J173	150 4X			Rx 4		Rx 12		Rx 15	
	200 9X	154 9	4 68	140 1	6 36	131 1	4 48	143 8	4 86
	300 3X								
J180	150 6X			Rx 5		Rx 9			
	200 4X	138 7	5 90	139 3	5 04	140 3	6 32		
J209	150 6X			Rx 5		Rx 9			
	200 4X	158 7	6 40*	154 0	5 58	97 8	5 77		
J108	150 3X			Rx 4		24 hr post Rx 7			
	200 4X	157 4	5 44	137 6	6 9	145 9	5 78		
J200	150 3X			Rx 4		Rx 7			
	200 4X	149 0	5 11	136 13	5 86	141 2	4 79		

\* To see serum K<sup>+</sup> values that are higher than normal for the drug (3.7 to 6 mEq/l) may reflect the fact that these dogs were relatively recent arrivals in the laboratory and the sedating patterns had not yet established.

† Rx represents number of doses following which blood was withdrawn for analysis.



In the cat nerve muscle preparation there was evidence of a direct effect of high doses of chlorpropamide on contractile force (FIGURE 10). Mono synaptic (knee jerk) and polysynaptic spinal reflexes (flexor reflex) in cats were decreased gradually; however, all these effects were not sufficiently marked to explain the degree of weakness observed in the intact animals.

HYPOGLYCEMIC EFFECT OF A SINGLE  
ORAL DOSE OF CHLORPROPAMIDE  
100 mg/kg IN RATS

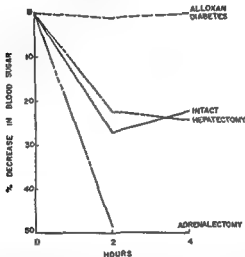


FIGURE 9 The effect of single oral doses of 100 mg/kg chlorpropamide to rats under various conditions. Blood sugar determinations were made every 2 hours. Fasting conditions were maintained throughout the test. Six rats were used in each group and the blood sugar responses of the groups were compared at each point with the preinjection level. Alloxan diabetic rats showed no hypoglycemia; adrenalectomized animals responded with a large drop in blood sugar followed by death between 2 and 4 hours after drug administration. No significant difference was observed in hepatectomized and intact animals.

performed in cats immobilized by change in doses of as much as 50 mg/kg by acoustic or sciatic stimulation were not abolished. Only after doses of 150 mg/kg I.V. or more was a slowing of the EEG noted, spindles were observed occasionally, but incon-

FIGURE 8 Effect of chlorpropamide on beta cell granules of rat pancreas stained with

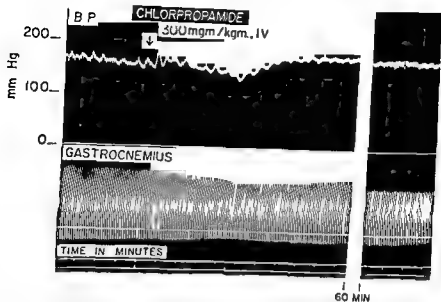


FIGURE 10 The effect of high doses of chlorpropamide on contractile force of the cat sciatic nerve gastrocnemius preparation (Nembutal anesthesia). The muscular contraction was reduced only by about 20 per cent following intravenous administration of high doses of chlorpropamide.

sistently. During these experiments, the blood pressure varied from 110 to 130 mm Hg, and showed only a slight rise after drug administration.

### Discussion

Although the hypoglycemic effect of chlorpropamide could be demonstrated in rats, rabbits, and dogs, the rhesus monkey was not only most sensitive to the drug but, like the human being, showed a more pronounced and more prolonged hypoglycemic response than it did to tolbutamide. The metabolic fate of chlorpropamide may vary from species to species and may explain these quantitative differences. Such species variations in metabolism for tolbutamide are known to exist between dogs and human beings.<sup>11</sup>

A cumulative effect may occur in dogs for the first few days following oral administration at various dose levels, but an equilibrium was reached within a few days beyond which cumulation did not progress. As observed by

■  
1"

in the human being

From the limited data available on the fate and excretion of chlorpropamide in dogs only, few conclusions can be drawn at this time. The long duration of action of chlorpropamide appears to parallel a slow rate of excretion.

Chromatographic analysis of urine will determine whether chlorpropamide

■ metabolized in the dog, unchanged excretion is reported for the human being.<sup>11</sup> The difference in hypoglycemic potency between dogs, on the one hand, and human beings and monkeys, on the other, may depend upon differences in metabolism. It has been suggested<sup>4</sup> that chlorination of the phenolic group prevented carbonylation of the compound in the para position, a reaction known to take place with tolbutamide in the human being.<sup>11</sup>

When given chronically to rats, dogs, and monkeys chlorpropamide was tolerated without untoward side effects in doses at least twenty times the recommended human dose. This represents a remarkable degree of safety for a powerful hypoglycemic agent. Of special interest was the lack of histopathological changes in livers of rats and dogs treated with high doses of chlorpropamide for periods of up to twenty months. These results are in agreement with findings of Root,<sup>4</sup> and they support the view that reversible jaundice, encountered only very rarely in human patients, is more a matter of individual idiosyncrasy than a toxic effect characteristic for chlorpropamide. It may be necessary to consider such factors as history of liver disease, impaired liver function prior to medication, or even increase in biliary secretion as described by Purnell *et al*<sup>14</sup> for the sulfonylureas.

Growth inhibition as seen exclusively in the rat experiments may have various causes and may eventually offer an approach to the mode of action of chlorpropamide. However, the results available at present do not allow an analysis of this drug effect. Metabolic interference of chlorpropamide with cysteine and/or glycine supply to the growing organism was considered, but this reaction is unlikely because addition of these amino acids to the diet did not overcome growth depression. It should be remembered that nonspecific stress<sup>15</sup> might be a factor.

sodium infusions than to glucose alone is yet unexplained, as no evidence of sodium deficiency could be obtained in these animals.

In itself the direct effect of chlorpropamide on the contractile force of striated muscle was not definite enough to account for ataxia and weakness. The same was true for the reflex depression obtained in cats.

Gerstenberg *et al*<sup>17</sup> had observed reflex depression in rabbits with tolbutamide, an effect that seemed to be much more pronounced and was assumed to be unrelated to hypoglycemia.

It is doubtful whether the EEG findings following high single doses of chlorpropamide offer an explanation for the side effects observed with toxic doses of the drug. Amounts of chlorpropamide necessary to elicit these EEG changes must be considered excessive. However, it is conceivable that hypoglycemia as well as central and peripheral nervous system effects

chlorpropamide are contributing to the symptoms described for the intact organism

### Summary

Chlorpropamide is a potent orally active hypoglycemic agent of long duration of action. Its activity may be demonstrated best in rhesus monkeys where it shows activity in the dose range used in human beings. Fate studies in dogs indicate slow excretion of the compound in acute experiments.

Upon chronic administration to various species of animals, chlorpropamide was found to be well tolerated in doses twenty times greater than the recommended human dose. With the exception of growth retardation in very high doses in rats, the compound produced no gross anatomical or histopathological changes.

Chlorpropamide is qualitatively similar to, but quantitatively more potent than, other hypoglycemic agents of the sulfonylurea types. Evidence was presented that certain central nervous system effects observed experimentally after high doses, or clinically following overdosage, may be related to a direct action of the drug rather than to its hypoglycemic effect.

### Acknowledgments

The work reported and summarized in this paper contains data obtained from studies performed by S Y Pan, J Kaiser, M Finkelstein, K Finger, J F Snell, L Schorr, A Drakontides, and their staff, whose efforts are greatly appreciated.

### References

- 1 SYMPOSIUM ON CLINICAL AND EXPERIMENTAL EFFECTS OF SULFONYLUREAS IN DIABETES MELLITUS 1956 *Metabolism* 5 721-977
- 2 THE EFFECTS OF THE SULFONYLUREAS AND RELATED COMPOUNDS IN EXPERIMENTAL AND CLINICAL DIABETES 1957 *Ann N Y Acad Sci* 71(1): 1-292
- 3 LOUBATIERES, A 1957 The hypoglycemic sulfonamides: history and development of the problem from 1942-1955 *Ann N Y Acad Sci* 71(1): 44
- 4 McLAMORE, W M, G M FANELLI, S Y PAN & G D LAUBACH 1957 Hypoglycemic sulfonylureas *Ann N Y Acad Sci* 74(1): 443-448
- 5 SALGADO, E D, Biological studies Meeting Endoc
- 6 ROOT, M A 1957 (chlorpropamide)
- 7 FOLIN, O & H I 1928 blood sugar det
- 8 SHIFTER, S, S DAYTON, B NOVIC & I MENIWEILER 1957 glyco- gen with the anthrone reagent *Arch Biochem* 25 191
- 9 TOOLAN, T J & R L WAGNER, JR 1957 The physical properties of chlorpropamide and *Ann N Y Acad Sci* 74(3): 449-458

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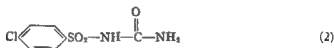
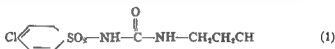
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- 12 CARLOZZI M, D G IZZONI & L SILVER Blood levels of chlorpropamide in normal men following chronic administration. *Ann N Y Acad Sci* 74(3)
- 13 JOHNSON P A, HENNES & K WEST The metabolic fate of chlorpropamide in man. *Ann N Y Acad Sci* 74(3) 459-470
- 14 PURNELL R, Y ABRAHAM I, PRATT C, HILAD JR & H FLICK 1956 Some observations on the mode of action of Orinase. *Metabolism* 5 778-787
- 15 SELYE H 1950 Stress 822. *Acta Inc Montreal Canada*
- 16 JAEGER D A Unpublished observation
- 17 GERSTENBERG L A, HASSELBLATT & G SCHMIDT 1957 Wirkung hoher Dosen des oralen Antidiabetikum D 860 (Artosin Rast non) auf neuromotorische Funktionen besonders des Rückenmarkes. *Arch exptl Pathol Pharmacol* 231 407-418

### *Discussion of the Paper*

MARY A ROOT (*The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.*) Jurg A. Schneider and I are apparently in complete agreement about the pharmacology of chlorpropamide.<sup>1</sup> However, I can add a bit to his study of its metabolism. All of this study of metabolism has been done by John Welles of our Toxicology Department.

Schneider mentioned that paper chromatography of urine from dogs given  $S^{35}$  labeled chlorpropamide yielded three spots. We have used unlabeled chlorpropamide in similar experiments and have also found three spots from dog urine. The spots were eluted from the paper, crystallized and then identified by x-ray diffraction and infrared studies. The formulas of these three substances are



The total amount of each metabolite was determined by elution from the paper and measurement of the ultraviolet absorption on a recording spectrophotometer. This method is not completely quantitative but does give approximate values.

In normal dogs maintained on a constant daily dose of chlorpropamide from 27 to 33 per cent of the drug (Formula 1) was excreted unchanged every 24 hours. The sulfonylurea (Formula 2) accounted for from 35 to 40 per cent of the administered dose, and the sulfonamide (Formula 3) for another 16 to 24 per cent. This gives a total 24 hour recovery of from 78 to 97 per cent of the daily dose. These studies do not exclude the possibility that there are other metabolites of chlorpropamide but Welles has been able to account for the major portion of the drug.

The metabolic products of both carbutamide and tolbutamide have been



shown to vary greatly from species to species<sup>2,4</sup> For this reason we have investigated the major metabolic products of chlorpropamide in rabbits. In these animals 80 to 95 per cent of a dose of chlorpropamide was excreted unchanged (Formula 1). Preliminary studies in man indicate that some of the drug is excreted unchanged but that there are probably 1 or 2 metabolic products. Much more work must be done before we can establish the existence of such products and if so their identity.

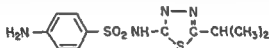
### *References*

1. ROOT, M. A., M. V. SIGAL, JR. & R. C. ANDERSON. 1959. *Diabetes* 8: 7.
2. ROOT, M. A. 1957. *J. Pharmacol. Exptl. Therap.* 119: 468.
3. LOUIS, L. H., S. S. FAJANS, J. W. COYNE, W. A. STRUCK, J. B. WRIGHT & J. L. JACKSON. 1956. *J. Am. Chem. Soc.* 78: 5701.
4. MOHNIKE, G., G. WITTENBOGEN & W. LANGENBECK. 1958. *Naturwiss.* 45: 13.

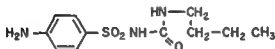
# HYPOGLYCEMIC SULFONYLUREAS: EFFECT OF STRUCTURE ON ACTIVITY

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*Research Laboratories Chas. Pfizer & Co. Inc. Brooklyn, N. Y.*

Although a very considerable literature has accumulated on the pharmacological and clinical properties of 2 or 3 sulfonylurea drugs, little or nothing has been published until quite recently on the effects of structural changes on hypoglycemic activity within this general class.<sup>1-4</sup> We thought it would be of interest, therefore, as background for this monograph, to outline the results of such a study which has been under way in our laboratories for some time and which has led to the hypoglycemic agent chlorpropamide (Diabinese). Moreover, although our results are in substantial agreement with those recently published, there are some minor differences.



2254 RP (IPTD)



CARBUTAMIDE (BZ-55)

FIGURE 1

The detailed history of the hypoglycemic sulfonylureas is too well known to require repetition here. One facet of this history, however, is pertinent to the present discussion, namely, the close structural resemblance (FIGURE 1) of the early sulfamylthiadiazoles (1942)<sup>5,6</sup> to the later sulfonylureas (1955).<sup>7</sup> In view of this close similarity it is perhaps surprising that the hypoglycemic effects of sulfonylureas were discovered so much later and apparently completely by chance.

Discovery of the hypoglycemic properties of 1-butyl-3-sulfamylurea or carbutamide<sup>7</sup> (BZ-55, Invenol, Nadisan) was soon followed by the discovery<sup>8,9</sup>

advantage of this structural change was the complete loss of antibacterial properties—a predictable result which was carefully confirmed for tolbuta

ratories and screened pharmacologically for their effect on the blood sugar

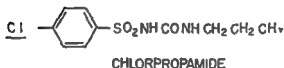
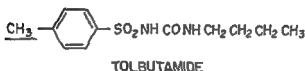
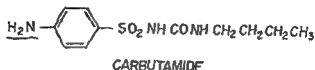


FIGURE 2

of fasting white rats at several intervals after oral administration of 100 to 300 mg/kg of the compound. Our goal of course was to find a drug of superior potency and duration of action and with minimal side effects. The structure activity correlations resulting from this study are outlined below.

As illustrated in FIGURE 3 the generalized sulfonylurea structure can be considered in three portions: (1) the alkyl chain  $R$ , (2) the urea portion, and

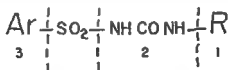


FIGURE 3

(3) the (substituted) aryl group. The effects on activity of structural changes in each of these three portions taken one at a time are indicated in FIGURES 4 to 8.

In most of the active series simple alkyl chains of 3 to 4 carbon atoms led to peak activity. Homologues having a branched alkyl chain such as *tert* butyl or a cyclic alkyl substituent had quite good activity with a probable shift of peak activity in the cyclic case to  $C_5$ - $C_7$ . Compounds





<u>R</u>	<u>ACTIVITY</u>
n-C <sub>3</sub> H <sub>7</sub> to n-C <sub>4</sub> H <sub>9</sub>	+++ to ++++
BRANCHED C <sub>3</sub> H <sub>7</sub> to C <sub>5</sub> H <sub>11</sub> (e g tert-C <sub>4</sub> H <sub>9</sub> )	++ to ++++
CYCLIC C <sub>5</sub> H <sub>9</sub> to C <sub>6</sub> H <sub>11</sub>	+++ to ++++
NHR = N $\begin{matrix} \text{R}_1 \\ \text{R}_2 \end{matrix}$ , e g -N 	++ to +++
R = aryl, e g  -Cl	++ to +++

FIGURE 4 Effect of the alkyl chain (R) on activity


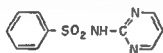
		<u>ACTIVITY</u>	<u>DURATION</u>
 -SO <sub>2</sub> -	$\text{NH} - \overset{\text{S}}{\overset{\parallel}{\text{C}}} - \text{NH} - \text{C}_4\text{H}_9$	++	+
	$\text{NH} - \overset{\text{O}}{\overset{\parallel}{\text{C}}} - \text{O} - \text{C}_4\text{H}_9$	+	
	$\text{NH} - \overset{\text{O}}{\overset{\parallel}{\text{C}}} - \text{CH}_2 - \text{CH}_3$	±	
	$\text{NH} - \overset{\text{O}}{\overset{\parallel}{\text{C}}} - \text{CH}_2 - \text{C}_4\text{H}_9$	±	
	$\text{NH} - \overset{\text{O}}{\overset{\parallel}{\text{C}}} - \text{C}_6\text{H}_5$	+	+
	$\text{NH-NH} - \overset{\text{O}}{\overset{\parallel}{\text{C}}} - \text{NH} - \text{C}_4\text{H}_9$	±	
	$\text{NH-NH} - \overset{\text{O}}{\overset{\parallel}{\text{C}}} - \text{C}_3\text{H}_7\text{-iso}$	±	
	$\text{N}(\text{CH}_3) - \overset{\text{O}}{\overset{\parallel}{\text{C}}} - \text{NH} - \text{C}_4\text{H}_9$	±	

FIGURE 5 Effect on activity of simple changes in the urea portion

in which the N' atom was disubstituted were moderately active. Replacement of the alkyl substituent by aryl also afforded some compounds of good activity.

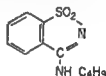
Modifications in the urea portion of the molecule that have been studied are outlined in FIGURE 5 (simple changes) and FIGURE 6 (more complex changes). Such modifications led almost uniformly to loss of activity; weak activity was retained in a few of the structural variants, notably the thiourea, the carbamate, and one of the more complex analogues.

Variations in the aryl portion of the molecule (FIGURES 7 and 8) were the most fruitful of all the structural modifications studied. In general *p* substituted aryl substituents led to compounds of maximal activity and duration of action. Unsubstituted phenyl derivatives were usually less active, *o*-substituted and *di* substituted (2,4, 2,5, 3,4) phenyl compounds were still

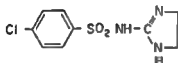


ACTIVITY

±

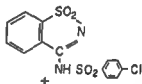


±



ACTIVITY

±



+

FIGURE 6 Effect on activity of more complex changes in the urea portion

less active. Naphthylsulfonylureas were very weakly active. Compounds in which the aryl group was heterocyclic (for example, 2-thienyl, 5-chloro-2-thienyl) were, however, moderately active.

The most effective of all *p* substituents studied was halogen (Cl, Br), and especially chlorine. From a considerable number of *p*-chlorobenzene-sulfonylureas with various alkyl substituents, the propyl compound was chosen on the basis of extensive animal work as the most potent and longest acting of all the sulfonylureas studied in our program. This compound has received the generic name chlorpropamide and the Pfizer trademark Diabinese.

Extensive further work in animals and in man has demonstrated this drug to be distinctly more potent and longer acting in all species than previous sulfonylureas. The relatively short duration of action of tolbutamide in man can very probably be attributed to its fairly rapid and complete metabolic

Chlorpropamide

This fact is in  
amide decrease








<u>Ar-SO<sub>2</sub>NHCO NH R</u>			
<u>Ar</u>	<u>R</u>	<u>ACTIVITY</u>	<u>DURATION</u>
Cl 	C <sub>3</sub> H <sub>7</sub>	++++	+++
(CHLORPROPAMIDE)			
Br 	C <sub>3</sub> H <sub>7</sub>	++++	+
F 	C <sub>3</sub> H <sub>7</sub>	++	++
CH <sub>3</sub> 	C <sub>4</sub> H <sub>9</sub>	+++	++
(TOLBUTAMIDE)			
	C <sub>4</sub> H <sub>9</sub>	±	
CH <sub>3</sub> 	C <sub>3</sub> H <sub>7</sub>	++	++
	C <sub>3</sub> H <sub>7</sub>	+++	++

FIGURE 7 Effect on activity of substitution in the aryl portion




<u>Ar-SO<sub>2</sub>NHCO NH R</u>			
<u>Ar</u>	<u>R</u>	<u>ACTIVITY</u>	<u>DURATION</u>
	α C <sub>4</sub> H <sub>9</sub>	±	++
	β C <sub>4</sub> H <sub>9</sub>	+	++
	C <sub>4</sub> H <sub>9</sub>	++	+
	C <sub>4</sub> H <sub>9</sub>	++	+

FIGURE 8 Effect on activity of more drastic changes in the aryl portion

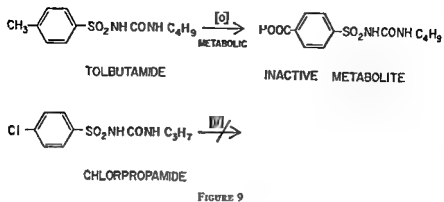


FIGURE 9

much less rapidly than those of tolbutamide and that the drug produces hypoglycemia of longer duration

References

1 CASSADY D R C AINS WORTH N R EASTON M LIVEZEY M V SIGAL, JR & E VAN HEYNINGEN 1958 J Org Chem 23 923  
2 MARSHALL F J & M V SIGAL JR 1958 J Org Chem 23 927  
3 HAACK E 1958 Arzneimittel Forsch 7(a) 444  
4 " " " " " " " " " " WAGNER & R WEYER 1958 Arznei  
5 1942 Montpellier méd 21 22 441  
6 ol 138 766 830  
7 Wochschr 80 1449  
8  
9 MASKE H 1956 Deut med Wochschr 81 823

# THE PHYSICAL PROPERTIES OF CHLORPROPAMIDE AND ITS DETERMINATION IN HUMAN SERUM

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*Chas Pfizer & Co Inc Brooklyn N Y*

Chlorpropamide [1 (*p* chlorobenzenesulfonyl) 3 propylurea : Diabinese\*] belongs to the class of arylsulfonylurea compounds, which have found wide spread use in the oral treatment of diabetes mellitus. It was felt worthwhile to study some of the physical properties of this compound and to develop a method suitable for its determination in human serum.

Chlorpropamide can be characterized by a number of physical properties. The present paper deals with preliminary observations on the following: (1) titration behavior, (2) melting point, (3) ultraviolet absorption spectrum, (4) infrared absorption spectrum, (5) solubility, (6) paper chromatography and (7) countercurrent distribution. Such data proved useful in the development of an assay method for this product.

Several assay methods have been reported for 2 other arylsulfonylurea compounds, namely, tolbutamide and carbutamide.<sup>1†</sup> Forst *et al*,<sup>2</sup> Spingler and Kaiser,<sup>3</sup> and Bladh and Norden<sup>4</sup> developed methods for the determination of tolbutamide (1 butyl 3 *p* tolylsulfonylurea); these were based essentially on the intense ultraviolet absorption of this compound at 228 m $\mu$ . In addition, McDonald and Sawinski<sup>5</sup> and Spingler<sup>6</sup> described colorimetric procedures for the determination of tolbutamide. Carbutamide (1 butyl 3 sulfantylurea) can be determined in serum by diazotization and coupling reactions.<sup>7</sup> The method to be described for chlorpropamide is based on the ultraviolet absorption of this compound at 232.5 m $\mu$  in 0.01 N hydrochloric acid. It is isolated from serum by a two stage extraction procedure which includes (1) extraction of serum acidified with phosphoric acid with chloroform, and (2) re-extraction of the chloroform phase with a dilute solution of sodium carbonate. The aqueous phase from the second extraction is adjusted to pH 2 with hydrochloric acid; the chlorpropamide content is determined by measurement of the absorbance at 232.5 m $\mu$ .

## EXPERIMENTAL

### *Physical Properties of Chlorpropamide*

**Titration.** Chlorpropamide is an acid; it can be titrated as such in several solvent mixtures. Dioxane-water, acetone-water, and U.S.P. ethanol-water were found to be quite useful for this purpose; equal volumes (10 ml.) of each solvent were utilized in these mixtures. Chlorpropamide (70 mg.) was dissolved in the dioxane-water medium and was then titrated potentiometrically with standard 0.5 N sodium hydroxide. The resulting titration curve (FIGURE 1) is that of a monobasic acid. A blank titration was also run

\* Diabinese is the trademark of Chas. Pfizer & Co. Inc., for chlorpropamide.

† These compounds are referred to in the literature under various designations and names (Constam<sup>8</sup>).



on the solvent mixture alone. An equivalent weight of 279 was found, that calculated for  $C_{10}H_{13}N_2O_3SCl$  is 276.7. In dioxane water a  $pH_{1/2}$  value of 6.5 was indicated for chlorpropamide, whereas the  $pH_{1/2}$  values in acetone water and U.S.P. ethanol water are 6.2 and 6.1, respectively. The  $pH_{1/2}$  value corresponds to the  $pH$  at the 50 per cent neutralization point in these titration curves. These determinations were made with a Beckman Model G  $pH$  meter.

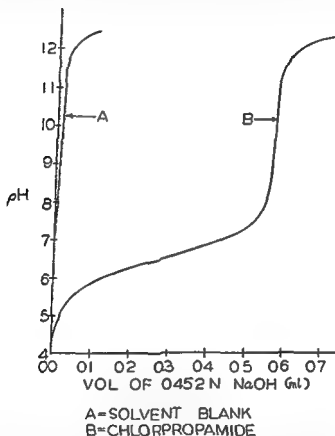


FIGURE 1 Potentiometric titration of chlorpropamide in dioxane water

**Melting point** Chlorpropamide melts at 127.5° to 128.5° C. Previously Marshall and Sigal<sup>4</sup> reported a range of 127° to 129° C, and Ruschig et al.<sup>5</sup> a range of 126° to 128° C.

**Ultraviolet absorption spectra** In 0.01 N hydrochloric acid chlorpropamide exhibits an ultraviolet absorption maximum at 232.5  $m\mu$  ( $\epsilon = 16.5 \times 10^3$ ) and a series of less intense absorption maxima through the 250 to 280  $m\mu$  wave length region. The latter occur at approximately 257.5  $m\mu$ , 265.5  $m\mu$ , 269  $m\mu$  and 277  $m\mu$ , with  $\epsilon = 710$  at 265.5  $m\mu$  (FIGURE 2). Absorbance at 232.5  $m\mu$  follows Beer's law over the concentration range 0-15  $\gamma/ml$ .

In USP ethanol, chlorpropamide shows a maximum at 231.5  $m\mu$  ( $\epsilon = 15.8 \times 10^3$ ) and a series of weaker absorption maxima through the 250 to 280  $m\mu$  wave length region. The latter occur at approximately 258  $m\mu$ , 265  $m\mu$ , 268.5  $m\mu$  and 276.5  $m\mu$  with  $\epsilon = 580$  at 265  $m\mu$ .

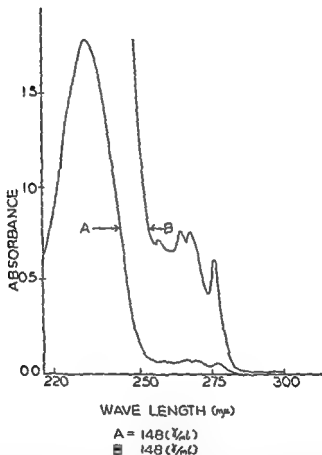


FIGURE 2 Ultraviolet absorption spectrum of chlorpropamide in 0.01 N HCl

In 0.01 N sodium hydroxide chlorpropamide exhibits an absorption maximum at 228.5  $m\mu$  ( $\epsilon = 13.2 \times 10^3$ ) absorption in the 250 to 280  $m\mu$  region is not as clearly defined as in the above two solvents.

*Infrared absorption spectra* Characteristic absorption maxima in the infrared spectrum of chlorpropamide between 2  $\mu$  to 15  $\mu$  are shown in FIGURE 3. These spectra

multi a potas

Solubility

solvents. Concentrations of 25 mg/ml are readily obtainable at room

particularly in acid media. The solubility of chlorpropamide in buffered (MacIlvaine) aqueous solutions at pH 2.2 to 6.0 was determined after 2 hours' equilibration at 25° C. The amount of this compound that dissolves in this pH range is as follows: pH 2.2, 0.1 to 0.2 mg/ml; pH 3.0, 0.1 to 0.2 mg/ml; pH 4.0, 0.1 to 0.2 mg/ml; pH 5.0, 0.3 to 0.4 mg/ml; pH 6.0, 2.1

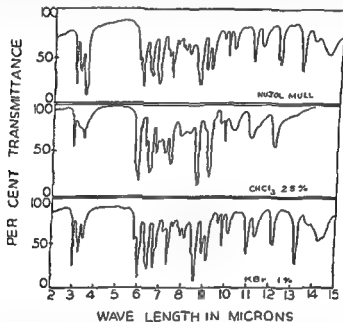


FIGURE 3. Infrared absorption spectra of chlorpropamide

to 2.3 mg/ml. At pH values greater than 6.5 concentrations of chlorpropamide greater than 5 mg/ml are readily obtainable under these conditions.

Thin layer chromatography was used to

An apparatus similar to that described previously with the following solvent systems:

(1) mobile phase, ethyl acetate-chloroform (1:3) saturated with formamide—immobilized phase, formamide-methanol (1:1), Whatman No. 1 paper, (2) 2 per cent  $K_2HPO_4$  with untreated Whatman No. 4 paper, and (3) mobile phase, chloroform saturated with ethylene glycol—immobilized phase, ethylene glycol-methanol (1:1), Whatman No. 4 paper. The 2 per cent  $K_2HPO_4$  system was used with ascending solvent flow. Solutions of chlorpropamide were spotted on paper in amounts containing 50 to 100  $\gamma$  of compound, at times as much as 1000  $\gamma$  was streaked on the paper. After development and drying, spots

were detected either by their ultraviolet absorption through a zinc silicate-coated fluorescent screen<sup>21</sup> or by spraying with blue tetrazolium reagent<sup>22</sup>. After spraying with the blue tetrazolium reagent, the sheet is warmed a short time in an oven (90° C), chlorpropamide exhibits a whitish zone on a general blue background. Single zones were observed in these systems. In the

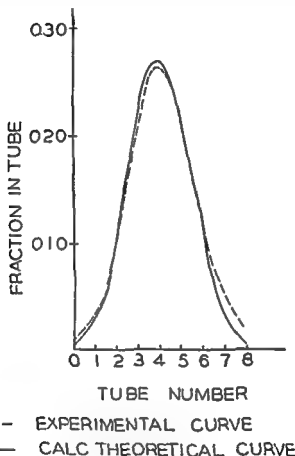


FIGURE 4 Countercurrent distribution of chlorpropamide between chloroform and pH 6.7 buffer

ethyl acetate-chloroform (1:3) system the  $R_f$  value was approximately 0.1 in the 2 per cent  $\text{K}_2\text{HPO}_4$  system, approximately 0.8-0.9, and in the chloroform-ethylene glycol system, approximately 0.2.

*Countercurrent distribution.* The homogeneity of chlorpropamide was tested by means of the technique of countercurrent distribution. This compound was distributed between chloroform and Clark and Lubs pH 6.7

**buffer** Under these conditions the distribution coefficient of chlorpropamide is about 1. An 8 plate transfer was run, using a chloroform solution of chlorpropamide (concentration of 300  $\gamma$ /ml). At the end of the distribution the chlorpropamide content of each phase in each tube was determined through ultraviolet spectrophotometry. When the fraction of the total chlorpropamide content per tube was plotted against the corresponding tube number, a typical distribution curve was obtained in good agreement with a calculated curve (FIGURE 4).

**Microanalytical data** Chlorpropamide can be recrystallized readily from acetone-water mixtures. Typical microanalytical data are calculated for  $C_{10}H_{13}N_2O_2ClS$ : C, 43.40, H, 4.74, N, 10.12, S, 11.59, Cl, 12.81. Found: C, 43.48, 43.45, H, 4.75, 4.84, N, 10.17, 10.02, S, 11.99, 11.84, Cl, 12.6.

#### *Determination of Chlorpropamide in Human Serum*

**Reagents** Five reagents were used in this study:

(1) 0.067 M Phosphoric acid: 4.55 ml of concentrated phosphoric acid (Mallinckrodt, A. R., 85 per cent) diluted to a total volume of 1000 ml with water.

(2) Chloroform: Mallinckrodt, A. R.

(3) One per cent sodium carbonate: Mallinckrodt, A. R., 10 gm of anhydrous sodium carbonate per 1000 ml of solution.

(4) Hydrochloric acid: (a) 0.4 N (b) 0.01 N.

(5) Chlorpropamide stock solution: 200 mg of chlorpropamide are slurried with 30 ml of distilled water and sufficient 0.1 N NaOH is added (with stirring) until a pH of 7 is reached. The resulting solution is transferred quantitatively to a 100-ml volumetric flask and diluted to the mark with distilled water. It is stable for at least one week when stored at room temperature. This stock solution serves as a convenient starting point in the preparation of solutions for use in recovery experiments and the determination of the  $E_{254}^{1\%}$  value of chlorpropamide.

**Apparatus** In this experiment the following apparatuses were used: (1) centrifuge tubes of 10- and 40-ml capacity, glass-stoppered and graduated, (2) a centrifuge capable of handling the centrifuge tubes, and (3) a spectrophotometer, either the Beckman Model DU spectrophotometer or the Cary Model 14 recording spectrophotometer quartz cells (1 or 2 cm) for absorbance measurements.

It should be remembered that the serum must be deproteinized by heating out this assay mixture and dried prior to use.

Chloroform

**Procedure** One ml of serum is mixed with 2 ml of 0.067 M phosphoric acid and 4 ml of water in a 40-ml centrifuge tube. The resulting acidified serum is then extracted with 10 ml of chloroform by gentle shaking for about 15 seconds\*. The phases are separated by centrifugation, and 5 ml of the

\* Occasionally an emulsion forms at this stage which is not easily broken by centrifugation. If this occurs an additional 10 ml of chloroform are added to the centrifuge tube and the contents are reshaken and centrifuged. Instead of a 5 ml aliquot a 10-ml aliquot of the clear chloroform layer is taken and the experiment continued as above.

chloroform layer is transferred to a 10-ml centrifuge tube containing 5 ml of 1 per cent sodium carbonate. The contents of this centrifuge tube are shaken and then centrifuged. A 4 ml aliquot of the sodium carbonate layer is transferred to a 10-ml volumetric flask, neutralized with 2 ml of 0.4 N hydrochloric acid and diluted to the mark with 0.01 N HCl. The absorbance value of this solution is determined at 232.5 m $\mu$  using either the Beckman DU spectrophotometer or the Cary spectrophotometer. The reference solvent is 0.01 N hydrochloric acid. As a blank a serum sample (devoid of chlorpropamide) is processed through this same procedure and measured in the same fashion. If a Cary recording spectrophotometer is available it is well occasionally to record the ultraviolet spectra of the sample and blank over the 220 m $\mu$  to 300 m $\mu$  wave length interval.

*Calculation.* Chlorpropamide concentration

$$(\gamma/\text{ml}) = \frac{(I_s - A_B)_{232.5\text{m}\mu}(10^4)}{(l)(E_{1\%}^{1\text{cm}})(4)}$$

where  $l$  is the cell path in centimeters,  $I_s$  and  $I_B$  are the absorbance values of serum sample and blank, and  $E_{1\%}^{1\text{cm}}$  is the absorbance of a solution of chlorpropamide in 0.01 N hydrochloric acid at a concentration of 1 gm per 100 ml when measured in a 1 cm cell at 232.5 m $\mu$ .

*Recovery experiment.* Known amounts of chlorpropamide (50 to 250  $\gamma/\text{ml}$ ) were added to human serum and then were processed through the above assay procedure. The results of a typical recovery experiment are shown in TABLE 1. Here recovery is essentially quantitative. Experiments on a

TABLE 1  
RECOVERY OF CHLORPROPAMIDE ADDED TO HUMAN SERUM

Chlorpropamide added ( $\gamma/\text{ml}$ )	Chlorpropamide found ( $\gamma/\text{ml}$ )	Recovery (percentages)
250	248	99
250	249	99
250	248	99
125	127	102
125	123	98
125	123	98
50	51	102
50	50	100
50	51	102

variety of serum samples to which chlorpropamide had been added in varying amounts (37 to 564  $\gamma/\text{ml}$ ) yielded a recovery of  $99 \pm 1.4$  per cent\*. It is recommended that recovery experiments be run on a regular basis to familiarize one with the method and to determine the  $E_{1\%}^{1\text{cm}}$  under laboratory conditions.

\* Average  $\pm$  average deviation.

*Serum blanks* A serum sample obtained from the same subject before the administration of chlorpropamide when processed through the normal procedure serves as a convenient serum blank. A variety of serum samples (free of chlorpropamide) from both diabetic and nondiabetic subjects was carried through the above extraction procedure and gave apparent chlorpropamide concentrations of  $13 \pm 2.7 \gamma/\text{ml}$ .

*Reproducibility experiment* Serum samples from several patients being treated with chlorpropamide were combined. Ten individual determinations were made on this pooled serum sample. These results are summarized in TABLE 2.

TABLE 2  
REPRODUCIBILITY OF ASSAY ON COMBINED SERUM SAMPLES

Assay No	Chlorpropamide content ( $\gamma$ ml)
1	191
2	194
3	191
4	193
5	194
6	191
7	198
8	191
9	195
10	194
	—
	193 $\pm$ 1.8

#### *Identification of Chlorpropamide in Human Serum*

In order to demonstrate the presence of chlorpropamide in human serum the following isolation experiment was conducted. Serum samples from 16 diabetic subjects were pooled to provide 30 ml of serum with a chlorpropamide content of  $138 \gamma/\text{ml}$ . This pooled serum sample was acidified to pH 2 to 3 with 0.067 M phosphoric acid and was extracted with chloroform

now shaken with a volume of 1 per cent sodium carbonate such that a fourfold increase in concentration was effected. This 1 per cent sodium carbonate layer was removed, neutralized with 0.4 N HCl and diluted so as to provide two solutions comparable in concentrations with those shown in FIGURE 2. The ultraviolet absorption spectra of these 2 solutions were recorded on a Cary Model 11 spectrophotometer and were found to be identical with those of chlorpropamide. After the determination of the ultraviolet absorption spectrum these solutions were combined and extracted with chloroform. The chloroform extracts were concentrated to dryness in a stream of nitrogen and the residue was dried at reduced pressure over potassium hydroxide.

The infrared spectrum of this residue was determined in chloroform in a microcell on a Baird recording spectrometer and it was identical with that of chlorpropamide. Furthermore the paper chromatographic behavior of this residue was the same as that of chlorpropamide in the 2 per cent  $\text{K}_2\text{HPO}_4$  system.

**Interferences** The method described for the determination of chlorpropamide is not specific for this compound. In order to test for possible interferences the following compounds were added to 1 ml of serum in the quantities indicated and were carried through the usual extraction procedure:

(1) *p*-chlorobenzenesulfonamide (0.25 mg)

(5)  $\beta$ -hydroxybutyric acid (0.5 mg)

(7) salicylic acid (0.5 mg). The ultraviolet absorption spectra of the resulting solutions were examined in the wavelength interval 220 to 350  $\text{m}\mu$  using the Cary Model 11 recording spectrophotometer. In the case of sodium *p*-chlorobenzenesulfonate, acetone and ethyl acetoacetate the resulting spectra were comparable to that of a serum blank. However the ultraviolet absorption spectrum of the solution obtained from the  $\beta$ -hydroxybutyric acid experiment exhibited strong end absorption thus making it difficult accurately to determine chlorpropamide in the presence of 5.5 mg/ml of this compound. With *p*-chlorobenzenesulfonamide, acetylsalicylic acid and salicylic acid major absorption maxima were found at 228  $\text{m}\mu$ , 227  $\text{m}\mu$  and 235.5  $\text{m}\mu$  respectively indicating that these compounds would present a serious interference in the procedure used to determine chlorpropamide.

#### SUMMARY

Chlorpropamide is an acidic substance as demonstrated by potentiometric titration. Its ultraviolet absorption spectrum shows a maximum at 232.5  $\text{m}\mu$  in 0.01 N hydrochloric acid with less intense maxima in the 250 to 280  $\text{m}\mu$  wave length region. The infrared absorption spectrum of chlorpropamide is characterized by a number of strong absorption bands through the 2 to 15  $\mu$  wave length region. It is soluble in a variety of common organic solvents but exhibits a limited solubility in water at pH values below 5. Its homogeneity can be demonstrated by paper partition chromatography and counter current distribution.

A method is presented for the determination of chlorpropamide in human serum. It consists of a two stage extraction procedure that includes an extraction of acidified serum with chloroform followed by re-extraction of the chloroform phase with dilute sodium carbonate. After suitable acidification of the sodium carbonate phase the chlorpropamide content is determined by measurement of the absorbance at 232.5  $\text{m}\mu$ . Recoveries of chlorpropamide from human serum and serum blank values are quite acceptable. The manipulations involved in the assay are relatively simple and determinations can be carried out in reasonably short time intervals. Chlor



$\beta$  hydroxybutyric acid  $p$  chlorobenzenesulfonamide acetylsalicylic acid and salicylic acid would present interferences

# ACKNOWLEDGMENTS

We acknowledge our sincere appreciation to T M Lees G W Walsh F R LaPietra R J Hickey W H Boegemann and H J Pazdera of the Chas Pfizer & Co Inc staff for their contributions to the work described in this paper

# REFERENCES

- 1 CONSTAN G R 1957 Sulfonyleurea compounds in the treatment of diabetes Northwest Med 88 1155-1166
- 2 FORIST A V J KRAKE W L MILLER & W A STRICK 1957 Determination of plasma levels of tolbutamide (1 butyl 3  $p$  tolylsulfonyl urea Oximase) Proc Soc Exptl Biol Med 98 180-183
- 3 SPINGLER H & F KAISER 1956 The determination of N (4 toluenesulfonyl) N butylurea in serum Arzneimittel Forsch 6 760-762
- 4 BLADH E & A NORDEN 1958 A method for determining 1 butyl 3  $p$  tolylsulfonylurea (tolbutamide) in human blood serum Acta Pharmacol Toxicol 14 188-194
- 5 McDONALD H J & V J SAWINSKI 1957 Simple colorimetric test for tolbutamide Abstract of Papers 132nd Meeting Am Chem Soc 1957
- 6 SPINGLER H 1957 Determination of N (4 methylbenzenesulfonyl) N butylurea in serum J Biol Chem 222 535
- 7 HANSEN J 1957 Determination of N (4 methylbenzenesulfonyl) N butylurea in serum J Biol Chem 222 535
- 8 MILLER W L 1957 Determination of N (4 methylbenzenesulfonyl) N butylurea in serum J Org Chem 23 927-929
- 9 RUSCHIG H G HORGER W AUMILLER H WAGNER R WYER A BANDER & J SCHOLE 1958 New orally effective blood sugar lowering substances Arzneimittel Forsch 8 448-454
- 10 BLAYTON R B A ZAFFARONI & E H LEUTMANN 1951 Paper chromatography of steroids II Corticosteroids and related compounds J Biol Chem 188 763-771
- 11 DRAKE N A W J HAINES R E KNAPP & E D NIELSON 1956 The ultra violet scanner camera for paper chromatography Anal Chem 28 2036-2038
- 12 PAZDERA H J W H McMULLEN L I CIACCIO & R MISSAL & T C GREENFELL 1957 Quality control of pharmaceuticals Anal Chem 29 1649-1654

## METABOLIC FATE OF CHLORPROPAMIDE IN MAN\*

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We have investigated the urinary excretion, blood levels, protein binding, and chromatographic behavior of chlorpropamide, a new sulfonylurea. This was accomplished by administering this drug to nondiabetics and to diabetic patients. Work by others has shown that tolbutamide, another sulfonylurea, has a serum half time of 4 hours and that it is carboxylated at the  $\beta$  methyl group *in vivo*.<sup>1,2</sup> On the other hand, carbutamide has a longer serum half time of 33 hours and the  $\beta$  amino group is acetylated *in vivo*.<sup>3</sup> To our knowledge there are no published reports concerning the metabolic fate of chlorpropamide in man.

### Methods

The radioactive chlorpropamide used in these experiments had an initial specific activity of 66.6 mg/mc. It was given as a water solution with sufficient carrier added so that 20  $\mu$ c of sulfur 35 equaled 250 mg of chlorpropamide. This solution was given to patients as follows:

**Group 1** Four nondiabetic patients without renal disease were given a single oral dose of 250 mg of radioactive chlorpropamide containing 20  $\mu$ c of sulfur 35.

**Group 2** Three stable diabetic patients, subject No. 1, 45 years old, received 500 mg tolbutamide twice daily; subjects Nos. 2 and 3, 71 and 64 years old, respectively, received 250 mg of chlorpropamide daily as therapy. On the day of the test each patient received orally one dose of 250 mg of radioactive chlorpropamide. No further chlorpropamide was given during the test period.

**Group 3** (a) A 41 year old diabetic patient who had not received chlorpropamide prior to this test was given intravenously 20  $\mu$ c of radioactive chlorpropamide without added carrier. One tablet of 250 mg of chlorpropamide was given orally at the same time. (b) A 71 year old diabetic was treated in the same way as the patients of Group 1.

The radioactive samples were counted with an automatic micromil gas flow GM tube with an average background of 14 cpm, using a preset count of 1000. Sufficient counts were obtained to give a 5 per cent average counting error or, in the case of the weaker samples, a sufficient number of counts to obtain at least 3 count rates that were within 1 cpm of the median count rate. The mean of these median values was considered to be the true count rate. Each sample was weighed and corrected for self absorption. All serum samples were deproteinized by sodium tungstate and sulfuric acid precipitation prior to plating.

\* The work reported in this paper was supported in part by a grant from Chas. Pfizer & Co. Inc., Brooklyn, N. Y.

The binding of chlorpropamide in serum was determined as follows: 200  $\mu$ g of chlorpropamide were added to 1 ml of serum. These aliquots were incubated at 37° C for 24 hours and then dialyzed at pH 7.6 for 48 hours. The per cent of the radioactivity remaining at the end of the dialysis was considered as the percentage of bound chlorpropamide. The binding of 4 types of protein solutions was determined as follows: (1) normal serum, (2) commercial serum albumin in 2 concentrations, 25 and 50 mg/ml, and (3) serum obtained from a patient with marked hypoalbuminemia. His serum protein was 4.7 gm per cent. Electrophoretic analysis showed that 2.6 per cent of this protein was albumin, 6.5 per cent alpha 1 globulin, 17 per cent alpha 2 globulin, 23 per cent beta globulin, and 51 per cent gamma globulin. *In vivo* binding was determined by giving a diabetic patient 250 mg of radioactive chlorpropamide orally. Serum samples were obtained at 4 and 48 hours. These samples were dialyzed in the same way as the protein solutions.

The chromatographic method used for the detection of chlorpropamide in blood and urine was a modification of the method of Seligson and Shapiro for  $\alpha$  keto acids.<sup>3</sup> The only significant change was elimination of the incubation with 2,4 dinitrophenylhydrazine. A chlorpropamide standard was used to locate the chlorpropamide spots under ultraviolet light after chromatography. Numerous unidentified spots seen under ultraviolet light after chromatography of extracts of urine are unrelated to the administration of chlorpropamide.

To determine whether erythrocytes absorbed chlorpropamide, we incubated 25-ml samples of fresh whole blood at 37° C for 2 hours in siliconized vessels gassed with 5 per cent CO<sub>2</sub> and 95 per cent O<sub>2</sub>. The plasma was separated by centrifugation. The erythrocytes were resuspended and washed in cold normal saline 4 times prior to the determination of radioactivity.

The serum concentration of chlorpropamide was measured by the technique of Toolan and Wagner.<sup>4</sup> This method makes use of a chloroform extraction of acidified serum with 0.067 M phosphoric acid. The chloroform extract is then washed with aqueous 1 per cent sodium carbonate. The aqueous sodium carbonate is acidified to pH 2 with HCl, and the optical density is measured at 232.5 m $\mu$ . The optical density of the fasting serum is subtracted and the net optical density is compared to a standard. The average optical density of the serum of 5 normals who had not received this drug previously gave an apparent chlorpropamide value of 17  $\mu$ g/ml by this method.

### Results

TABLE 1 contains the serum radioactivity values obtained from 4 non-diabetic patients following the ingestion of 250 mg of chlorpropamide containing radioactive sulfur 35. Maximum serum radioactivity and optical

The log of the  
meets the mean  
The first and

more rapid of these has an uncorrected half time of 32 hours, the slower component has a half time of approximately 16 days. However, since only 2 data points were obtained, this value is only a rough estimate. If the effects of the slower component are removed from the initial slope, a half time of approximately 24 hours is obtained. These data show that the serum

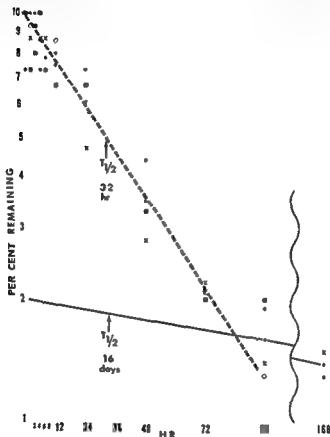


FIGURE 1 Disappearance of serum radioactivity from the serum of 4 nondiabetic patients. In this figure, 10 is equal to 100 per cent.

disappearance of chlorpropamide occurs at 2 separate rates. Half times determined from these data are:

rst 3 subjects  
The fourth

TABLE 1  
SERUM RADIOACTIVITY CORRECTED TO PEAK ACTIVITY

Disappearance of radioactive chlorpropamide from the serum of 4 nondiabetic patients  
The maximum serum radioactivity has been made equal to 100 The actual  
maximum serum radioactivity is listed at the bottom of the table

Hours	Subject No				Mean
	1	2	3	4	
1	100	100	73	100	
2	87	93	100	73	
4	93	93	100	80	
6	87	86	73	100	86
8	87	73	73		78
12	67	86	80	67	75
24	47	57	73	67	61
48	28	36	44	33	35
72	21	21	21	20	21
96	14	13	19	20	16
168	15		13		14
Net cpm/ml serum at 100%	90	84	90	81	

TABLE 2  
SERUM RADIOACTIVITY CORRECTED TO PEAK ACTIVITY

		Group No 2			Group No 3	
Subject No		1	2	3	1	2
Hours						
1		100	55	93	100	82
2		85	100	100	76	100
3					92	
4		85	64	111		100
5					76	
6		100	64	75		82
8		85	73	83	72	70
24		77	54	50	64	59
48		69	45	42	40	41
72		46	36	25	28	35
96		54	36	25	20	35
Net cpm/ml serum equal to 100%		60	66	72	150	72

subject received this drug intravenously and the fifth subject received this drug in the same way as the patients of Group I the data from this table are shown in FIGURE 2. It can be seen that the early disappearance of the radioactivity from the serum in all but one subject was similar to that of the nondiabetic patients given this drug. However, all values fall outside of 2

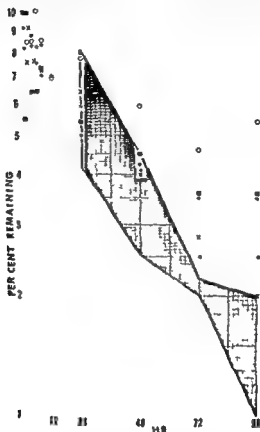


FIGURE 2. Disappearance from the serum of radioactive chlorpropamide in 5 diabetic subjects. In this figure 10 is equal to 100 per cent.

standard deviations from the mean of the normal group after the 48-hour specimen. Whether this higher percentage of slowly excreted chlorpropamide represents differences between the handling of the drug in normals and diabetics cannot be determined from these data since the groups are not comparable. It is notable that the disappearance of the radioactivity from the serum of subject No. 1 was much slower than that of the other subjects. This diabetic patient preloaded with tolbutamide had a normal phenol sulfonphthalein excretion study and blood urea nitrogen. His serum

uric acid level was elevated but there was no other evidence of gout except that he had had an episode of pain in the right ankle of several days' duration on one occasion 2 months prior to these studies

TABLE 3 presents the percentage of the ingested dose excreted in the urine of the nondiabetic subjects in Group 1. The patients are presented in the same order as they were in TABLE 1 so that a comparison can be made between

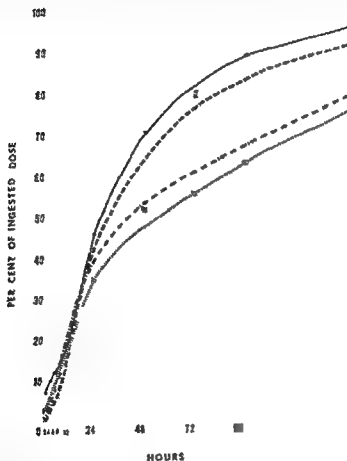


FIGURE 3 Cumulative urinary excretion of radioactive chlorpropamide in 4 nondiabetic patients

the serum and urinary radioactivity. FIGURE 3 shows these data plotted linearly. The mean cumulative excretion during the 96 hours of the tests was 77 per cent. This agrees well with the 77 per cent values which showed that 16 per cent of the radioactivity was excreted in the urine 96 hours after the ingestion of the dose in the patients without gout.

TABLE 3 presents the percentage of the ingested dose excreted in the urine

TABLE 3  
CUMULATIVE URINARY EXCRETION OF RADIOACTIVE CHLORPROPAMIDE BY FOUR  
NONDIABETIC SUBJECTS

The values presented are the percentages of the ingested dose

Subject No	1	2	3	4
Hours				
2	0.7	1.3	7.1	2.6
4	2.7	3.6	10	5.2
6	4.9	8.4	12	7.8
8	6.4	11	13	12
12	17	21	19	17
24	42	41	46	35
48	60	54	71	52
72	80	62	81	56
96	81	63	90	64

TABLE 4  
CUMULATIVE URINARY EXCRETION OF RADIOACTIVE CHLORPROPAMIDE BY FIVE  
DIABETIC SUBJECTS

The results are presented as percentages of the ingested dose

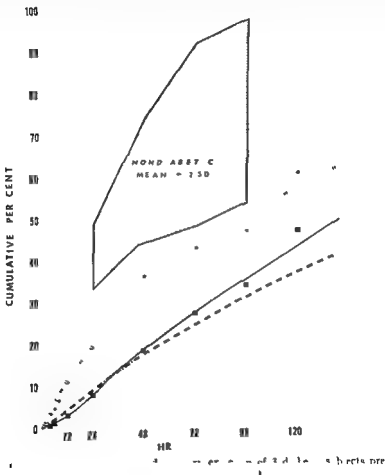
Subject No	Group No 2			Group No 3	
	1	2	3	1	2
Hours					
2	0.4		1.2	3.0	3.9
4	1.7	0.5	3.4	8.2	5.1
6	3.0	1.3	4.7		13
8	3.7		6.8	12	15
12	4.5	3.0	11	17	22
24	8.5	7.8	19	36	35
48	17	11	36	66	55
72	24	27	43	88	66
96	31	34	47	92	75
120		47	61		79

obtained from the diabetic patients of Groups 2 and 3. Their serum values are presented in the same order as in TABLE 2. In FIGURE 4 the urinary excretion of these preloaded patients was markedly less than that of the normal group. However, it should be noted that the 2 groups are not completely comparable in regard to certain other factors such as age and the presence of diabetes. Consequently, the differences in the preloaded group and the group not loaded prior to testing could be due to other factors. The mean cumulative urinary excretion of the nondiabetics of Group 1, plus and minus 2 standard deviations is presented on this figure for comparison.

FIGURE 5 shows the cumulative urinary excretion of the other 2 diabetic



patients of Group 3. The patient with the highest urinary excretion received this drug as therapy up to the day of the test. On the day of the test he received an intravenous tracer dose of chlorpropamide without added carrier followed by a 0.25 gm. tablet of chlorpropamide. His rapid excretion of the drug is markedly different from that of the other 3 diabetics. The other excretory curve was obtained from a patient given 1 oral dose of this drug.



His excretory pattern is very similar to that obtained from the 4 patients in the normal group.

Fecal excretion of an intravenously injected chlorpropamide was determined in 1 diabetic patient who was already taking this drug as therapy. Carmine red dye given at the same time as the tracer dose appeared in his 48-hour stool specimen. The patient's fecal excretion of chlorpropamide was 0.52 per cent on day 1, 0.92 per cent on day 2, and 0.74 per cent on day 3.

It would appear that fecal excretion is not a very significant excretory pathway.

TABLE 5 shows the results of our dialysis experiments. *In vitro* binding by a normal subject was 6.9 per cent. A patient with a total serum protein of 4.7 gm per cent with almost no albumin showed binding of 3.1 per cent which is less than one half of that of the normal serum. Albumin solutions

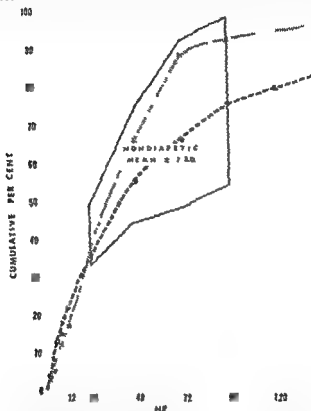


FIGURE 5. Cumulative chlorpropamide urinary excretion of 2 diabetic subjects. The mean  $\pm 2$  S.D. of the mean of the normal group is presented for comparison.

of 2.5 and 7.0 gm per cent bound 2.2 and 4.0 per cent respectively. These data suggest that both albumin and globulin participate in this binding. However, no complete serum fractionation studies have been done. We did not find binding of chlorpropamide by crystalline insulin solutions of 1 to 3 mg/ml (32 units/mg). These concentrations of insulin are considerably less than those of the other protein solutions; therefore binding of a degree equal to that of albumin could have been missed. Nondiabetically chlorpropamide was present in the serum of a diabetic patient who had ingested this drug. These values approximate those of the *in vivo* experiments.

TABLE 5

PROTEIN BINDING OF RADIOACTIVE CHLORPROPAMIDE MEASURED BY DIALYSIS  
Determined by dialyzing serum protein samples at pH 7.6 for 48 hours

	Per cent bound
<i>In vitro</i>	(Mean $\pm$ standard error)
Normal	5.87 $\pm$ 0.66
Hypoalbuminemic	3.08 $\pm$ 0.15
Albumin 2.5 gm %	2.23 $\pm$ 0.08
Albumin 5.0 gm %	4.60 $\pm$ 0.33
<i>In vivo</i>	
Diabetic 4 hours	4.35 $\pm$ 0.60
Diabetic 48 hours	6.96 $\pm$ 0.99

TABLE 6

EFFECT OF INCREASING CONCENTRATIONS OF CHLORPROPAMIDE ON PROTEIN BINDING  
Each sample was incubated for 24 hours at 37° C and dialyzed at pH 7.6 for 48 hours

Concentration ( $\mu$ g/ml)	Normal serum		Serum albumin (50 mg/ml)	
	Per cent	$\mu$ g	Per cent	$\mu$ g
25	7.8	1.9	6.1	1.5
50	6.6	3.3	6.6	3.3
100	4.9	4.9	4.9	4.9
200	1.3	26	1.1	22

In an attempt to determine the effect of varying concentrations of chlorpropamide on these binding phenomena, we added 25 to 200  $\mu$ g of chlorpropamide to the serum samples. Table 6 shows that as the concentration of

With the chromatography system used by us the sulfur-35 labeled chlorpropamide standard exhibited 3 separate radioactive areas. Little difference was noted in the  $R_f$  values of the standard and that obtained from serum and urine specimens. The  $R_f$  values obtained are shown in TABLE 7. Seventy to 85 per cent of the radioactivity was associated with the  $R_f$  value 0.9 in the standard and in the extracts of urine and serum. This area of radioactivity corresponds to the major area of ultraviolet light absorption

The incubation of chlorpropamide with blood resulted in a plasma radio-

TABLE 7  
CHROMATOGRAPHY OF SULFUR-35 LABELED CHLORPROPAMIDE

Chromatography of serum and urine specimens following the ingestion of chlorpropamide. Peak No. 1 contained 70 to 85 per cent of the radioactivity, Peak No. 2 contained 10 to 25 per cent of the radioactivity, and Peak No. 3 contained 0 to 5 per cent of the radioactivity.

Peak No	1	2	3
Chlorpropamide standard	0.9	0.4	0.2
2 Hour serum	0.8	0.5	
8 Hour serum	0.9	0.3	
2 Hour urine	0.9	0.4	0.2
8 Hour urine	0.9	0.4	

activity of 15 730 net counts per minute (ncpm) and an erythrocyte radioactivity of 70 ncpm. After 2 hours of incubation the plasma count was 16 120 ncpm, and the erythrocyte count was 100 ncpm. This slight difference in radioactivity in the erythrocytes could be accounted for easily by experimental variations in the washing of the erythrocytes. In confirmation of these data, erythrocytes obtained from a patient given an oral dose of radioactive chlorpropamide failed to show absorbed radioactivity.

### Discussion

The disappearance of radioactive chlorpropamide from the serum of the 4 nondiabetic patients in Group 1 showed at least 2 separate disappearance rates. These dual disappearance phenomena exhibited by chlorpropamide

not been reported to occur following the ingestion of carbutamide and tolbutamide.

Extrapolation of the slower component back to zero time suggests that approximately 20 per cent of chlorpropamide could be present as this slowly excreted component; this is 3 times greater than values obtained in the binding experiments. Whether this slower component merely represents chlorpropamide bound to serum proteins or metabolized chlorpropamide cannot be determined from these data. Our evidence would suggest that this slowly

shows that significant differences in the time required to excrete this drug exist among diabetic patients receiving this drug as therapy.

The urinary excretion of radioactive chlorpropamide closely parallels its disappearance from the serum. The excretion of chlorpropamide by the preloaded diabetic patients was not as uniform as that of the nondiabetic patients. This would suggest that significant differences exist in the excre-

tion of this drug by various diabetic patients. Because of the possible importance of these differences and because this may be related to the disease state further study should be made of this phenomenon. Unlike carbutamide or tolbutamide this compound does not seem to be metabolized prior to excretion since no definite chromatographic differences were noted in the serum, urine and standard.

Binding of carbutamide and tolbutamide by serum proteins has not been reported. Chlorpropamide appears to be bound to both serum albumin and globulin. This binding is related to the concentration of chlorpropamide present in the serum and approximately equal binding can be produced *in vitro*. The presence of binding by proteins may account for the odd distribution space determinations obtainable after ingestion of this drug. By determining the space of distribution of intravenously administered chlorpropamide in two 70 kg diabetic patients 10 and 11 l spaces were obtained. These values do not correspond to either the extracellular or intravascular space and correcting it for amount of chlorpropamide bound by the serum proteins still fails to produce a space of distribution comparable to that reported for carbutamide or tolbutamide. We can surmise that binding to proteins other than serum proteins or leakage into the cells must occur. Failure to find absorption of chlorpropamide by *in vitro* incubation of erythrocytes does not rule out concentration of this drug by other tissues.

### Summary

(1) We have used chlorpropamide labeled with sulfur 35 to determine the metabolic fate of this drug.

(2) The disappearance of chlorpropamide has an initial apparent half-life of 32 hours for the major portion of the dose and a half-life of 16 days for the remainder. Chlorpropamide is excreted almost entirely by the kidneys. Only very slight fecal excretion was demonstrated.

(3) Chlorpropamide is made partially nondialyzable by serum proteins. Increasing the concentrations of chlorpropamide in the serum increases binding.

(4) We could not detect metabolic alteration of chlorpropamide prior to excretion.

### Acknowledgments

The technical assistance of Thomas Redding, Ronald Lee and Beverly Wulff and the art work of Shirley Shipman are greatly appreciated.

### References

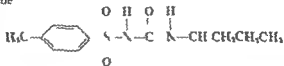
1. STOWERS, J. M. & F. MAHLER & R. B. HUNTER. 1958. Pharmacology and mode of action of the sulfonylureas in man. *Lancet* 1: 278.
2. LOUIS, L. H. & S. S. FAJANS, J. W. COYNE, W. A. STRICK, J. M. WRIGHT & J. L. JONES. 1936. The structure of a urinary excretion product of 1-butyl-3- $\beta$ -tolyl sulfonylurea. *J. Am. Chem. Soc.* 58: 51.
3. SELIGSON, D. & B. SHAPIRO. 1952. Alpha keto acids in blood and urine studied by paper chromatography. *Anal. Chem.* 24: 754.
4. TOOLAN, T. J. & R. L. WAGNER, JR. 1959. The physical properties of chlorpropamide and its determination in human serum. *Ann. N. Y. Acad. Sci.* 74(3): 449.

*Discussion of the Paper*

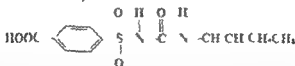
STEFAN S IJAZS (*University of Michigan, Ann Arbor Mich*) In view of the almost complete excretion of chlorpropamide in urine reported by Philip Johnson I shall mention briefly some published and unpublished observations about the metabolism and excretion of tolbutamide

In 1956 we reported upon the findings of a major urinary excretion product of tolbutamide after its administration to normal and diabetic subjects. Upon acidification of the urine of such subjects to a pH 5 or less a white precipitate appeared. This was shown to be the carboxylic acid derivative of tolbutamide (1 butyl 3 *p* carboxyphenyl sulfonylurea) as reported by us in conjunction with Struck and others of the Upjohn Research Laboratories Kalamazoo Mich

Tolbutamide



Tolbutamide excretion product



On very large dosages of tolbutamide such as 6 gm per day a maximum of about 75 per cent of the administered drug could be recovered from the urine as this metabolic oxidation product. This was determined by acidifying the urine and then drying and weighing the purified insoluble precipitate.

In order to rule out additional urinary metabolites of tolbutamide and to determine more quantitatively the urinary excretion of the carboxyl compound a joint project was set up with W L Miller of the Upjohn Laboratories at his suggestion. It was planned to give  $^{14}\text{C}$  tolbutamide to normal and diabetic subjects and to examine the urine for metabolites of tolbutamide by paper chromatography.

As can be seen from TABLE 1 3 gm of unlabeled tolbutamide was given daily to 6 subjects for 5 days and 15 mg of  $^{14}\text{C}$  tolbutamide was administered on the fourth day. Urines were collected for 24 hours on the fourth and fifth day. The chromatographic studies were performed by W L Miller.

The results of these studies are given in the table. It is apparent from the data for the first 4 subjects (3 control subjects and 1 tolbutamide non-re-positive diabetic subject) that 90 per cent or more of the administered drug was excreted as the carboxyl sulfonylurea within the first 24 hours and nearly 100 per cent within 48 hours. Furthermore the carboxyl excretion product was the only human transformation product of tolbutamide identified.

The lesser amounts of radioactivity recovered in the urine of the last two diabetic patients could well be accounted for by unfortunate errors in urine collection on the hospital wards. In these two patients there was also no evidence of excretion of a metabolite other than the carboxyl substituted tolbutamide.

In summary, it can be stated that

(1) the carboxylic acid derivative of tolbutamide is the only urinary excretion product in man, and (2) on the basis of data from the first four subjects, it appears that tolbutamide is quantitatively excreted in the urine as this metabolite.

TABLE 1  
URINARY EXCRETION OF  $S^{35}$  CARBOXYL SULFONYLUREA AFTER ORAL ADMINISTRATION OF  $S^{35}$  TOLBUTAMIDE\*

Subjects	$S^{35}$ Excretion, per cent		
	0 to 24 hours	24 to 48 hours	0 to 48 hours
Normal control			
T D	99.0	1.0	100.0
G R	95.8	4.3	99.9
J D	89.8	10.0	99.8
Tolbutamide nonresponsive diabetic			
R J	90.0	9.0	99.0
Tolbutamide responsive diabetics			
R C	—	—	90.8
N M	73.1	6.9	80.0

\* Tolbutamide was administered 3 gm. per day for 5 days.  $S^{35}$  tolbutamide, 15 mg. on fourth day. Urine collections were made on fourth day (0 to 24 hours) and fifth day (24 to 48 hours).

## RELATIVE POTENCIES OF CHLORPROPAMIDE AND TOLBUTAMIDE IN MAN

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Administration Hospital, Oklahoma City, Okla.*

In a previous publication<sup>1</sup> we have reported the results of experiments in which the hypoglycemic potencies of chlorpropamide and tolbutamide in 10 normal subjects were compared at varying intervals after administration of the drugs by mouth. One gram of chlorpropamide produced responses that exceeded those to 1 gm. of tolbutamide at 1, 2, 3, 4, 6, 12, and 18 hours after administration. More recently the venous blood glucose levels<sup>2</sup> of 20 normal subjects were determined 1 hour after each subject had received 1 gm. of tolbutamide on one occasion, and after 1 gm. of chlorpropamide on another occasion at intervals of 1 week. The order in which the drugs were administered was alternated. The drugs were administered orally in tablet form together with 4 ounces of water. The mean difference between the control fasting blood glucose values and the blood glucose levels 1 hour after tolbutamide averaged 66 mg. per cent, while chlorpropamide produced responses averaging 141 mg. per cent. This difference was highly significant ( $p < .001$ ). Since it seems unlikely that either drug had been appreciably metabolized or excreted as early as 1 hour after administration by mouth, the greater potency of chlorpropamide is probably not due entirely to its slower rate of degradation and excretion. The fact that chlorpropamide was definitely more potent 1 hour after administration suggests the possibility that it is more potent than tolbutamide even when serum levels are comparable. However, the levels of the drugs in the serum were not determined in this experiment. Consequently, it is possible that the greater potency of chlorpropamide is partially due to the fact that it is more readily or more completely absorbed.

Figure 1 shows the responses of 6 normal subjects to varying doses of chlorpropamide and tolbutamide when the blood glucose levels were measured 2 hours after the doses were administered in random order at intervals of not less than 4 days (those subjects who received 2 gm. of chlorpropamide were not given another dose for at least 1 week). Each of the subjects received 4 different doses of each drug with the exception that only 3 of the subjects received a dose of 2 gm. of chlorpropamide. In each instance the fasting blood glucose was compared to the blood glucose 2 hours after a drug.

It may be noted that with each of the doses the responses were greater to chlorpropamide than to tolbutamide. Tolbutamide 0.25 gm., produced no response while 0.5 gm. of the drug produced responses comparable to those after 0.25 gm. of chlorpropamide. Tolbutamide, 1 gm., and chlorpropamide, 0.5 gm., also produced similar responses. It is of interest that tolbutamide 2 gm. produced responses that were not greater than those to 1 gm., which suggests the possibility that the point of maximum response was being



reached. However, the data are not adequate to establish this point. On the other hand, the responses to the various doses of chlorpropamide were roughly related to the log of the dose.

Since the biological half time of chlorpropamide is several times longer than that of tolbutamide (Johnson *et al*, in this monograph), it seems likely that differences in the responses to chlorpropamide and tolbutamide would be far greater than those demonstrated here if the effects were measured 12 to 48 hours after comparable doses of the 2 drugs.

That the high serum levels present for several days after a single dose of chlorpropamide are associated with proportionate physiological activity of

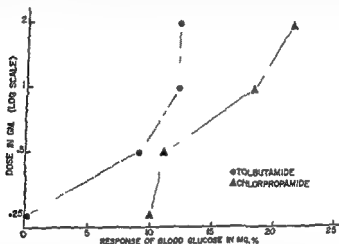


FIGURE 1 Hypoglycemic potencies of tolbutamide and chlorpropamide in normal subjects. Each plot represents the mean response of 11 subjects 2 hours after the oral administration of the drug except that only 3 of the subjects received 2 gm of chlorpropamide. The patients who received chlorpropamide also received tolbutamide. See text for details.

the drug is suggested by the following example. We administered chlorpropamide 2 gm to one of our normal subjects, whose blood glucose was measured 1 hour later and proved to be 44 mg per cent. At 2 hours it was

not be entirely aroused from sleep. The state of semiconsciousness persisted for 1 hour before being relieved immediately by the intravenous injection of 50 gm of glucose. The level of serum chlorpropamide at this time was 16.4 mg per cent.

#### Serum Levels of Orally Administered Chlorpropamide

Johnson *et al*, in this

some way partially bound to a substance or substances in the plasma. Elsewhere in this volume Johnson *et al.* indicate that chlorpropamide is to some extent bound to protein. If this is the case it is probably not appropriate to refer to a chlorpropamide space. If however it were assumed that the drug was homogeneously distributed in a space the data in TABLE 1 would indicate that such a space would necessarily be less than 9 l in volume since there is at least 115 mg in every liter of plasma 2 hours after oral administration of 1 gm of the drug.

### *The Effect of Chlorpropamide on Glucose Tolerance*

One of the strongest arguments against the direct or indirect peripheral action of the sulfonylurea agents has been the failure to demonstrate an effect of single doses of tolbutamide on glucose tolerance.<sup>4, 5</sup> On the other hand TABLE 2 shows that chlorpropamide produced in each of 5 subjects a statistically significant increase in the rate of disappearance of intravenously administered glucose. Venous blood glucose determinations were carried out 10

the test was repeated in the same manner except that chlorpropamide 0.5 gm was injected with the glucose. The control mean disappearance rate was 1.22 per cent per minute. After chlorpropamide the mean disappearance rate was 1.85 per cent per minute. One of these subjects weighing 178 pounds had a control rate of 1.6 per cent per minute which increased 2.4 per cent per minute after chlorpropamide 0.5 gm. It is of interest that the

TABLE 1  
SERUM LEVELS OF CHLORPROPAMIDE AFTER 1 GM ORALLY

Subject	Weight (lbs.)	Serum chlorpropamide	
		1 Hour (mg %)	2 Hours (mg %)
1	155	3.6	13.0
2	145	5.8	10.9
3	124	2.9	10.6
4	118	6.9	12.0
5	185	8.1	12.7
6	114	7.0	10.3
7	145	8.2	10.7
Mean	149	6.1	11.5

intravenous administration to this subject of 1  $\frac{1}{2}$  units of glucagon free insulin produced a strikingly similar change (2.5 per cent per minute)

### DISCUSSION

It is not possible to establish accurately the ratio of the potencies of chlorpropamide and tolbutamide from the data in FIGURE 1. In the first place, too few subjects have been tested to establish precisely a dose response curve for either drug. However, it is clear that in a certain range of dosage chlorpropamide produced an effect approximately comparable to that produced by doses of tolbutamide twice as large. As mentioned previously, it may be assumed that the difference in the responses to chlorpropamide and tolbutamide on a milligram for milligram basis could be expected to be much greater if the tests were done from 12 to 48 hours after administration of the drugs because of the longer biological half time of chlorpropamide (Johnson *et al*, in this monograph). The difference in potency might be expected to be particularly marked if the response to the drugs were compared after daily administration in which case there would be a cumulative effect for a few days with chlorpropamide and no appreciable cumulative effect with tolbutamide.

It should be stressed that the subjects who received chlorpropamide (FIGURE 1) were the same who received tolbutamide. This paired-control design will, of course, make it possible to identify significant differences in the dose response curve with a fewer number of tests since the factor of the varying responsiveness from subject to subject is eliminated. At present we are doing a paired-control study in which responses of normal subject to 0.25 gm chlorpropamide are being compared to their responses to 0.5, 1, and 2 gm of tolbutamide. This experiment will continue until statistically significant differences are identified.

The degree to which studies of potencies of sulfonylurea derivatives in normal subjects are applicable to the establishment of their potencies in various

TABLE 2  
THE EFFECT OF 0.5 GM CHLORPROPAMIDE ON INTRAVENOUS GLUCOSE TOLERANCE

Fasting blood glucose (mg )		10 min after glucose* (mg )		40 min after glucose (mg )		Glucose disappearance rate " per minute†	
Control	Chlorprop	Control	Chlorprop	Control	Chlorprop	Control	Chlorprop
70	70	163	173	135	134	0.57	0.75
70	67	148	150	100	159	1.05	1.82
2	59	158	146	88	25	1.48	2.76
66	63	130	130	82	45	1.23	1.67
3	71	146	130	69	42	1.76	2.25
Mean 70	66	149	142	95	65	1.22	1.85
							P < 0.01

\* Fifteen gm injected in 1 to 2 min. timed from start of inject on

† Assuming that the per cent per minute disappearance during a 30-min period occurred at a constant rate.

types of diabetic subjects is not known. In general, however, we have found in studies with carbutamide, tolbutamide, chlorpropamide, and metahexamide that there has been a good correlation between the potencies of the drugs in normal subjects and their potencies in diabetics of the maturity-onset type. However, this matter has not been systematically evaluated by us, and the opinion expressed here is based only on our "clinical impressions" and requires verification by more direct studies.

#### *Summary and Conclusions*

Chlorpropamide, 1 gm, produced significantly greater hypoglycemic responses in normal subjects 1 hour after ingestion than did tolbutamide, 1 gm. Chlorpropamide 0.25 gm and 0.5 gm, produced hypoglycemic responses in normal subjects 2 hours after ingestion comparable to doses of tolbutamide twice as great. Since it is unlikely that either drug had been appreciably metabolized or excreted 1 to 2 hours after ingestion, the relatively greater potency of chlorpropamide probably is not attributable entirely to its longer biological half time.

Seven normal subjects had serum chlorpropamide levels averaging 11.5 mg per cent 2 hours after ingesting chlorpropamide, 1 gm, indicating that about one third of this dose was present in the plasma at that time.

Chlorpropamide 0.5 gm significantly increased the rate of disappearance of intravenously injected glucose. In one subject the effect of an intravenous injection of chlorpropamide 0.5 gm, on the glucose disappearance rate was strikingly similar to that of 1 R units of insulin administered intravenously.

Two findings that have resulted from studies carried out since this report

tolbutamide were administered intravenously, the hypoglycemic effects during the first hour were not significantly different.

#### *Acknowledgments*

The authors are indebted to B. J. Matter, Charles Key, Dorothy A. Wood and Robert Henry for technical assistance in carrying out these studies.

#### *References*

1. WEST, K. M. & S. R. McCAMPBELL. 1958. The hypoglycemic potency of a new sulfonylurea derivative (chlorpropamide) in man. *Proc. Soc. Exptl. Biol. Med.* **5**: 724-725.
2. NELSON, N. 1954. A photometric adaptation of the Somogyi method for determination of glucose. *J. Biol. Chem.* **163**: 375-380.
3. KENOLD, A. F., F. WENIGRAD, K. TROFSCH & G. W. THOMAS. 1956. Studies on the site of action of arylsulfonylureas in man. *Metabolism* **5**: 757-767.
4. FAJAN, S. S., J. H. LOUIS, H. S. SELTZER, R. D. JENNISON, R. D. GITTLE, A. R. HENNESSY, B. J. WACHSBERG, I. P. ACKERMAN & J. W. LOAN. 1956. Metabolic effects of arylsulfonylurea compounds in normal men and in diabetic subjects. *Metabolism* **5**: 820-839.
5. BASTENIE, P. A., A. CONARD & J. R. M. IRADIERSON. 1954. Effect of cortisone on carbohydrate metabolism measured by the glucose assimilation coefficient. *Diabetes* **3**: 215-219.

# EFFECTS OF ADMINISTRATION OF CHLORPROPAMIDE UPON THE CARBOHYDRATE METABOLISM OF ISOLATED TISSUES\*

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It has been shown previously that the administration of carbutamide to rats over a 14-day period can cause a slight increase in the level of glycogen in the diaphragm<sup>1</sup>. If this diaphragm is removed and incubated in a suitable medium there is a decrease in the glycogen level, and this decrease is greater in diaphragms from treated animals than in those from control animals. This larger drop is probably only a reflection of the higher initial level of glycogen, but the higher initial level is compatible with the theory that carbutamide acts by increasing the effective insulin level in the plasma, possibly by increasing insulin secretion by the pancreas. A similar amount of tolbutamide does not cause a statistically significant increase, although the experimental data suggest such an effect.

This type of experiment has been repeated using chlorpropamide, to learn whether similar increases in muscle glycogen could be obtained. The drug was made up as either a 2½ per cent or a 1¼ per cent suspension in 1 per cent methyl cellulose (Methocel) solution and was force fed daily to rats in measured amounts so that a dose of either 250 mg/kg/day, or 125 mg/kg/day was given. At the same time, Ornase† and RP 2259‡ were given to other rats in a dosage of 250 mg/kg/day. Control rats received only the Methocel. They were kept on this regime for from 5 to 7 days and were then sacrificed.

## Results

The rats used in these tests were divided into a group of adult rats (Group 1) and a group of younger rats that were still growing (Group 2). The average weight changes are shown in the first two lines of TABLE 1. Generally speaking the effect of the hypoglycemic agents is to cause a weight loss in the older animals, or a decreased weight gain in the younger ones.

The results of measurements on initial glycogen levels in the diaphragm, on glycogen decrease in the diaphragm after incubation in a saline phosphate medium, on glucose uptake of the diaphragm, on liver glycogen and glucose release by a liver slice, on plasma potassium and on plasma glucose are further shown in TABLE 1. Results from the 2 groups of animals are pooled, since there seemed to be no difference between the younger and the older animals.

An analysis of the variance shows that there is a significant difference

\* The work reported in this paper was supported in part by grants from Chas Pfizer & Co. Inc. Brooklyn N.Y. and from Poulenc Ltd. Montreal, Canada.

† Tolbutamide

‡ 2 Sulfamido *p*-aminobenzene *S*-tert-butyl 1-thio-3,4-diazole

between the values for initial glycogen Chlorpropamide (at its high level) and RP 2259 seemed to cause a small increase Neither Ornase nor chlorpropamide (at its low dose level) show such an effect

In a second experiment chlorpropamide was given 350 mg/kg day for 14 days If anything there was a drop in diaphragm glycogen level 2.99 mg/gm for controls 2.77 mg/gm for treated animals The average weight gain for treated animals was 34 gm, compared to an average gain of 83 gm for control animals The test animals were in a poorer nutritional state and this may account for their failure to show an increase in muscle glycogen

In additional experiment in which Ornase and RP 2259 were given,

value of 2.04 mg/gm which is not significantly greater than the control level

TABLE 1

	Control	RP 2259	Ornase	Chlorpropamide		No of animals
				1 1/4%	2 1/2%	
Weight changes (grams)						
Group 1	6.3	-6.3	-2.7	-8.0	-7.7	3
Group 2	25.0	25.3	12.0	19.7	21.7	3
Initial diaphragm glycogen (mg/gm)	2.75	3.65	2.57	2.57	3.27	5
Glycogen decrease from original level (mg/gm)	1.40	1.20	1.02	0.96	1.40	5
Liver glycogen (mg/gm)	9.8	8.6	9.4	9.3	12.4	5
Liver glucose production (mg/gm)	11.9	10.5	9.2	10.3	8.3	5
Glucose uptake (mg/gm)	1.64	2.01	1.46	1.51	1.32	3
Blood glucose (mg%)	140	140	135	143	125 (162)	6
Plasma potassium (mEq/ml)	6.18	6.11	6.37	6.45	5.99	4

The values for the first experiment (TABLE 1) show that there were no significant changes in glycogen losses in the diaphragm following incubation in glucose uptake by the diaphragm in liver glycogen or glucose production by liver. The values for blood glucose and sugar values show that the value shown for the value omitted. The average value with this peculiar high value included is shown in brackets. The experiments with the higher doses of the hypoglycemic agents showed that chlorpropamide caused a drop in liver glycogen to 57 per cent of the

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It has been shown previously that the administration of carbutamide to rats over a 14 day period can cause a slight increase in the level of glycogen in the diaphragm<sup>1</sup>. If this diaphragm is removed and incubated in a suitable medium there is a decrease in the glycogen level, and this decrease is greater in diaphragms from treated animals than in those from control animals. This larger drop is probably only a reflection of the higher initial level of glycogen, but the higher initial level is compatible with the theory that carbutamide acts by increasing the effective insulin level in the plasma possibly by increasing insulin secretion by the pancreas. A similar amount of tolbutamide does not cause a statistically significant increase, although the experimental data suggest such an effect.

This type of experiment has been repeated using chlorpropamide, to learn whether similar increases in muscle glycogen could be obtained. The drug was made up as either a 2½ per cent or a 1¼ per cent suspension in 1 per cent methyl cellulose (Methocel) solution and was force fed daily to rats in measured amounts so that a dose of either 250 mg/kg/day, or 125 mg/kg/day was given. At the same time, Ornase† and RP 2259‡ were given to other rats in a dosage of 250 mg/kg/day. Control rats received only the Methocel. They were kept on this regime for from 5 to 7 days and were then sacrificed.

## Results

The rats used in these tests were divided into a group of adult rats (Group 1) and a group of younger rats that were still growing (Group 2). The average weight changes are shown in the first two lines of TABLE 1. Generally speaking the effect of the hypoglycemic agents is to cause a weight loss in the older animals or a decreased weight gain in the younger ones.

release by a liver slice, on p. 4. A possible explanation for the results is further shown in TABLE 1. Results from the 2 groups of animals are pooled, since there seemed to be no difference between the younger and the older animals.

An analysis of the variance shows that there is a significant difference

\* The work reported in this paper was supported in part by grants from Chas. Pfizer & Co. Inc. Brooklyn N. Y. and from Poulenc Ltd. Montreal Canada.

† Tolbutamide

‡ 2 Sulfamido *p*-aminobenzene 5-tert-butyl-1-thio-3,4-diazole

between the values for initial glycogen. Chlorpropamide (at its high level) and RP 2259 seemed to cause a small increase. Neither Orinase nor chlorpropamide (at its low dose level) show such an effect.

In a second experiment chlorpropamide was given 350 mg/kg/day for 14 days. If anything there was a drop in diaphragm glycogen level 2.99 mg/gm for control, 2.77 mg/gm for treated animals. The average weight gain for treated animals was 34 gm compared to an average gain of 83 gm for control animals. The test animals were in a poorer nutritional state and this may account for their failure to show an increase in muscle glycogen.

An additional experiment in which Orinase and RP 2259 were given 400 mg/kg/day showed that after 3 days neither agent had any effect upon

value of 2.04 mg/gm which is not significantly greater than the control level.

TABLE 1

	Control	RP 2259	Orinase	Chlorpropamide		No of animals
				1½%	2½%	
Weight change (grams)						
Group 1	6.3	-6.3	-2.7	-8.0	-7.7	3
Group 2	23.0	25.3	12.0	19.7	21.7	3
Initial diaphragm glycogen (mg/gm)	2.75	3.65	2.57	2.57	3.27	5
Glycogen decrease from original level (mg/gm)	1.30	1.20	1.02	0.96	1.49	5
Liver glycogen (mg/gm)	9.8	8.6	9.4	9.3	12.4	5
Liver glucose production (mg/gm)	11.9	10.5	9.2	10.3	8.3	5
Glucose uptake (mg/gm)	1.61	2.01	1.56	1.53	1.32	3
Blood glucose (mg%)	150	150	135	143	125 (162)	6
Plasma potassium (mEq/ml)	6.18	6.11	6.37	6.45	5.99	4

The values for the first experiment (TABLE 1) show that there were no significant changes in glycogen losses in the diaphragm following incubation in glucose uptake by the diaphragm in liver glycogen or glucose production by the liver slice or in plasma potassium levels. Blood sugar values show that the average that



control value. Orinase caused a drop to 42 per cent of the control value, where RP 2259 caused a drop to 60 per cent of the control value.

### *In vitro Studies*

The influence of chlorpropamide *in vitro* on certain tissues was also studied. The glucose output of liver slices from normally fed rats was measured when this compound was added to the incubation medium in different concentrations. Slices were incubated in a Dubnoff shaker for 45 min at 37° C in 10 ml of phosphate saline buffer. The results are shown in TABLE 2.

TABLE 2

	Chlorpropamide concentration mg/ml			
	0.00	0.06	0.25	1.00
Glucose output mg/gm	9.45	8.91	9.65	9.92
Per cent change from control		-5.7	+2.1	+5.0

The inhibitory action of chlorpropamide on glucose output is evident at a concentration of the drug that is about the same as that reported in the blood of human subjects. This suggests that chlorpropamide may act at least in part, by inhibition of liver activity. No further studies were done to elucidate the reason for the increase in glucose output with a higher concentration of chlorpropamide.

Chlorpropamide can also inhibit the oxygen uptake of certain tissue. Kidney slices from normal rats were incubated in a Krebs-Ringer phosphate medium in a Warburg apparatus, and oxygen utilization was measured. After 1 hour chlorpropamide was added, and it was found that at a concentration of 0.1 mg/ml it gave a 19 per cent inhibition in the rate of oxygen uptake. At 0.01 mg/ml it gave a 14 per cent inhibition.

When added to a rat liver homogenate at a concentration of  $10^{-3}$  M (0.28 mg/ml) in the presence of cytochrome *c* and succinate<sup>2</sup> there was a drop of 7 per cent in the oxygen consumption rate, at  $10^{-2}$  M (2.8 mg/ml) a drop of 13 per cent was observed.

### *Conclusion*

Chlorpropamide may, under certain conditions slightly increase the glycogen content of the rat diaphragm. Under similar conditions Orinase has no effect. RP 2259 has a greater effect. Any stimulating action upon glycogen synthesis is consistent with the hypothesis that certain oral hypoglycemic agents act by increasing the plasma insulin activity.

Chlorpropamide in low concentration can cause a small reduction in the glucose output of liver slices and a reduction in oxygen utilization by kidney slices and liver homogenates. It is suggested that part of the hypoglycemic action of chlorpropamide may come about through its hepatic action.

*Acknowledgments*

We are indebted to Chas Pfizer & Co, Inc, for supplies of chlorpropamide and to Poulenc, Ltd for supplies of RP 2259

*References*

- 1 CLARKE, D W & H SENMAN 1958 Fourteen day administration of carbutamide tolbutamide and cortisone *Diabetes* 7 283
- 2 UMBREIT W W, H H BURRIS & J F STAUFFER 1945 *Manometric Techniques* 95 Burgess Minneapolis Minn

# THE EFFECT OF INSULIN AND CHLORPROPAMIDE ON CERTAIN LIVER ENZYMES IN RATS

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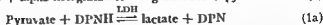
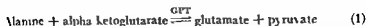
In the over all animal economy insulin is known to be protein sparing a property that has not as yet been associated with the newer oral hypoglycemic agents. The mechanisms by which insulin exerts its effects on protein metabolism are still unclear but it has been assumed that these effects are secondary manifestations of the alterations in carbohydrate metabolism known to occur in hypoinsulinism and hyperinsulinism. The present study was prompted by several reports<sup>1, 2</sup> wherein it was proposed that the gluconeogenic action of the adrenal corticosteroids is based on their ability to stimulate the hepatic activity of glutamic pyruvic transaminase (GPT) one of the two major transaminase systems involved in protein metabolism. The effect of insulin and chlorpropamide on GPT and glutamic-oxaloacetic transaminase (GOT) activity seemed worthy of study. The results presented below compare the effects of these two hypoglycemic agents on GPT and GOT activity with those of hydrocortisone. Lactic dehydrogenase (LDH) activity was also studied as an example of an enzyme system not directly concerned with protein metabolism.

## Experimental

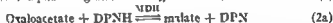
Eighty two albino rats weighing from 100 to 200 gm were used for this study. Food and water were offered freely unless otherwise indicated. Protamine zinc insulin and chlorpropamide (sodium salt) were administered in varying doses of from 1 to 4 U/kg and from 50 to 100 mg/kg, respectively. Hydrocortisone acetate was administered in a dosage of 5 mg per animal. With the exception of one preliminary acute experiment, all drugs were given subcutaneously in 7 daily doses over an 8 day period.

On the day of sacrifice all food and drugs were withheld. The animals were stunned and exsanguinated. The livers were removed immediately and portions were weighed for dry weight and enzyme assays. Those portions removed for assay were immediately frozen in a dry ice alcohol bath and stored at  $-20^{\circ}\text{C}$  prior to analysis. A 5 per cent homogenate of the sample for assay was prepared in the cold and suitable dilutions for each assay were prepared.

GPT activity was measured by a modification of the method of Wroblewski.<sup>3</sup> In this determination pyruvic acid formed in reaction 1 is reduced to lactate after the addition of excess LDH and in the presence of reduced diphosphopyridine nucleotide (DPNH).



GOT activity was measured by a modification of Karmen's procedure.<sup>4</sup> In this assay oxaloacetate formed by REACTION 2 is reduced to malate by an excess of malic dehydrogenase (MDH) and in the presence of DPNH as in REACTION 2a



LDH activity was determined by a modification of a procedure based on REACTION 1a.<sup>5</sup> The oxidation of DPNH in each of these reactions was followed spectrophotometrically at 340 m $\mu$ . One unit of activity for all enzymes represents a change in optical density of 0.001 O.D. units/min. Although all reactions were carried out within a fairly narrow temperature range, the results were corrected to a temperature of 25° C by a separately determined series of temperature correction factors, a procedure found desirable in previous studies.<sup>6,7</sup>

Aliquots of the original homogenates were analyzed for nitrogen by the micro Kjeldahl method. Although not always presented, results were calculated in terms of wet weight, dry weight, and nitrogen content.

### Results

Preliminary experiments were performed to ascertain the acute effect of the administration of insulin and chlorpropamide on liver enzyme activity. Three pairs of rats were fasted for 18 hours before use. Crystalline insulin (2 U/kg) and chlorpropamide (50 mg/kg) were administered intraperitoneally.

One pair of rats received only saline and served as a control. No significant differences in hepatic enzyme activity were found between the control and treated animals (TABLE 1) despite markedly depressed terminal blood sugars in the latter groups.

The absence of any change in this acute experiment is in line with what has been found in studies concerning the acute effects of insulin administration.<sup>8</sup> Hastings *et al.*<sup>8</sup> have concluded that insulin exerts immediate metabolic effects only at the periphery, and that its effect on hepatic metabolism is delayed and gradual. Further experiments reported herein were all extended to eight days.

#### *Effects of insulin, chlorpropamide, and hydrocortisone in rats fed ad libitum*

TABLE 2 presents the results of one experiment evaluating the effect of insulin (1 U/kg/day) and chlorpropamide (50 mg/kg/day) given in 7 injections over an 8 day period. The actual results for each animal are illustrated here to demonstrate the variability of individual measurements and to compare the results on the basis of wet weight, dry weight, or nitrogen

TABLE 1  
EFFECT OF ACUTE ADMINISTRATION OF INSULIN AND CHLORPROPAMIDE ON HEPATIC ENZYME ACTIVITY

Treatment	Enzyme activity U/mg dry wt		
	GPT	GOT	LDH
(a) No treatment	84.4	973	1420
(b) Insulin (regular), 4 U/kg	83.5	1000	1450
(c) Chlorpropamide (Na), 100 mg/kg	78.8	910	1315

TABLE 2  
VARIABILITY IN HEPATIC ENZYME ACTIVITY IN RATS

Treatment	Enzyme activity (units)								
	GPT			GOT			LDH		
	1*	2*	3*	1	2	3	1	2	3
(a) Control	19.3	64.7	609	213	715	6720	442	1490	13,900
	21.5	72.3	658	203	633	6230	438	1470	13,400
	23.1	74.5	663	217	764	6790	401	1300	11,600
Mean	21.3	70.5	644	218	704	6580	428	1417	13,000
(b) Insulin (PZI) 1 U/kg	12.5	39.5	359	211	666	6063	342	1080	9,827
	15.6	46.3	414	206	612	5461	399	1180	10,600
	15.6	48.1	400	207	648	5370	381	1170	9,900
Mean	14.6	44.6	391	208	642	5630	374	1140	10,100
(c) Chlorpropamide 100 mg/kg	19.3	58.8	553	197	601	5640	305	930	8,740
	16.9	54.0	469	199	636	5530	356	1140	9,900
	15.8	51.3	451	152	494	4350	419	1360	12,000
Mean	17.3	54.7	491	183	577	5180	360	1140	10,200

\* Columns 1, 2, and 3 represent units/mg wet weight, dry weight, and nitrogen, respectively.

content. Final evaluation of the effects of therapy were made on the combined data from this and subsequent experiments on 27 animals.

The results, shown in TABLE 3a indicate that hydrocortisone has a differing action on the 2 hepatic transaminases studied. Thus, although steroid administration apparently had no effect on GOT activity, it resulted in a marked and significant ( $p < 0.01$ ) increase in GPT activity. These latter

TABLE 3  
EFFECT OF THERAPY ON HEPATIC ENZYME ACTIVITY IN FED AND RESTRICTED ANIMALS

Treatment	Enzyme activity ( $\pm$ S.E.) U/mg dry wt		
	GPT	GOT	LDH
(a) Ad libitum feeding			
Control	67.4 ( $\pm 1.9$ )	701 ( $\pm 10$ )	1300 ( $\pm 25$ )
Chlorpropamide 50-100 mg/kg	55.8 ( $\pm 1.8$ )	565 ( $\pm 5.3$ )*	1090 ( $\pm 13$ )
Insulin (PZI) 1-4 U/kg	56.2 ( $\pm 1.7$ )	572 ( $\pm 7.1$ )*	1200 ( $\pm 16$ )
Hydrocortisone 5 mg	181 ( $\pm 8.6$ )*	689 ( $\pm 36$ )	1430 ( $\pm 88$ )
(b) Ad libitum feeding plus hydrocortisone			
Hydrocortisone alone	181 ( $\pm 8.6$ )*	689 ( $\pm 36$ )	1430 ( $\pm 88$ )
Hydrocortisone plus chlorpropamide 100 mg/kg	214 ( $\pm 4.2$ )*	584 ( $\pm 12$ )*	1200 ( $\pm 21$ )
Hydrocortisone plus insulin (PZI) 2 U/kg	195 ( $\pm 7.6$ )*	524 ( $\pm 33$ )*	1170 ( $\pm 29$ )
(c) Restricted feeding			
No treatment	125 ( $\pm 2.8$ )*	976 ( $\pm 22$ )	966 ( $\pm 14$ )*
Hydrocortisone	197 ( $\pm 3.7$ )*	755 ( $\pm 15$ )*	1090 ( $\pm 29$ )
Chlorpropamide 100 mg/kg	101 ( $\pm 2.8$ )*	857 ( $\pm 22$ )	1130 ( $\pm 37$ )
Insulin (PZI) 2 U/kg	110 ( $\pm 4.3$ )*	921 ( $\pm 31$ )	984 ( $\pm 35$ )*

\* Significantly different from control values ( $p < 0.01$ )

findings are in accord with numerous other reports on the effect of adrenocorticosteroids on the glutamic pyruvic transaminase<sup>1, 2, 10, 11</sup> Although GOT activity has been reported to be unaffected by similar therapy<sup>1, 11, 12</sup> in line with our present findings there have been conflicting reports<sup>2, 10</sup> This disagreement however may be a matter of degree, since it is the consensus that GOT activity is much less markedly influenced by steroids if at all than is GPT

In contrast to these findings the administration of insulin (1 to 4 U/kg/day) and chlorpropamide (50 to 100 mg/kg/day) were both shown to be accompanied by a significant decrease ( $p < 0.01$ ) in hepatic GOT activity as shown in TABLE 3a GPT activity although apparently somewhat depressed was not significantly affected ( $p > 0.18$ ) No reports of similar *in vivo* studies on the effects of insulin or chlorpropamide on hepatic transaminases have come to our attention However our observations would

seem to be in accord with the report by Copenhaver *et al*<sup>11</sup> that hepatic GOT activity is markedly elevated in alloxan diabetic rats

chlorpropamide on GOT activity was due to a direct hormonal influence on the liver or an indirect type of response due to changes in the periphery. Hydrocortisone together with either insulin or chlorpropamide was administered to a group of 17 animals to compare the effects of a combination of

day) resulted in changes in enzyme activity that reflected the influence of the steroid as well as the hypoglycemic agents. Thus GPT activity was increased as a result of hydrocortisone therapy despite the simultaneous administration of the hypoglycemic agents. As shown in TABLE 3a, GOT activity was depressed after the injection of insulin or chlorpropamide despite the administration of hydrocortisone. Lactic dehydrogenase activity

activity of both glutamic oxaloacetic and pyruvic transaminase<sup>12</sup>. The effect of hydrocortisone, insulin, and chlorpropamide on this increase in enzyme activity was studied in a group of thirty-two animals.

TABLE 3c shows that even the limited food restriction imposed in the present experiments was capable of enhancing the activity of the two transaminases under consideration. The administration of hydrocortisone to these animals resulted in a further augmentation of GPT activity beyond that produced by food limitation alone. GOT activity, on the other hand, was apparently decreased to those levels encountered in ad libitum fed animals. These findings further substantiate previous indications that hydrocortisone has a diverse effect on these two closely related enzymes. Contrary to the findings with animals on a free diet, neither insulin nor chlorpropamide could be shown to exert any influence on the activity of these enzymes.

by, however, resulted in a significant decrease ( $p < 0.01$ ) in the level of this enzyme in contrast to the increases observed in the level of both transaminases. It is interesting that limitation of dietary intake was the only

experimental procedure capable of altering the activity of the lactic dehydrogenase enzyme

### Discussion

The results obtained in this investigation must be interpreted with caution. Much additional work is needed to elucidate the role that these enzymes play in whole body homeostasis. One fact stands out, namely, that the action of chlorpropamide paralleled that of insulin in all experiments. The cause and physiological significance of these changes in hepatic enzymes, however, are more difficult to evaluate.

The early work of Bach and Holmes<sup>14</sup> showed that the addition of insulin to liver slices of normal fasted rats inhibited the transformation of certain amino acids into carbohydrates. Jensen and Gray<sup>15</sup> employing both *in vitro* and *in vivo* techniques found that insulin exerts an inhibitory effect on hepatic amino acid dehydrogenase activity in normal rats. In their *in vivo* experiments insulin administration led to a 25 per cent decrease and adrenal cortical extracts to a 205 per cent increase in the activity of this enzyme. Since Stad *et al.*<sup>17</sup> showed that the enzyme influenced by insulin affects only the unnatural D isomer of amino acids, the physiological importance of these findings is doubtful. However, it is interesting to note that a similar increase in GPT and a decrease in GOT activity were obtained in this study after hydrocortisone and insulin, respectively. This similarity would appear to throw some doubt on the view that the change in transaminase activity after steroid therapy is a reflection of specific hormonal control.

This latter concept was suggested by Rosen *et al.*<sup>4</sup> as an explanation of the marked increase in GPT activity and subsequent gluconeogenesis accompanying the administration of cortisone. The increase in enzyme activity after food restriction might be construed as a reflection of a stress induced oversecretion from the adrenal cortex. The differences in the action of insulin and chlorpropamide under the conditions of restricted and unrestricted feeding make this interpretation unlikely.

Starvation, however, like hydrocortisone therapy, is accompanied by an alteration in the metabolism of proteins toward catabolism. Insulin appears to stimulate anabolism. It has already been suggested that hepatic transaminase activity is inversely related to protein synthesis,<sup>10, 18</sup> a view that appears to accord with current findings. Similarly, Recant and Fischer<sup>9</sup> recently presented data indicating that tolbutamide, as well as insulin, stimulates the incorporation of tagged glycine into the liver proteins of starved male rats. These authors suggested that the effect of tolbutamide

changes in enzyme activity are under direct hormonal control or whether they represent a direct effect of the drug on the metabolic processes.

The findings of Zuchlewski and Gaebler<sup>21</sup> who demonstrated that the transfer of isotopic nitrogen from alanine to other amino acids occurred to the same



extent in the livers of normal and hypophysectomized rats treated with growth hormone despite a marked reduction in the GPT activity of the hormone treated animals. Similar studies on the effects of hydrocortisone, insulin, and chlorpropamide might yield a more definitive concept of the mechanisms by which these play a role in protein metabolism and its relation to gluconeogenesis.

### Summary

Insulin and chlorpropamide act similarly in moderately depressing glutamic oxaloacetic transaminase activity in the livers of well fed animals. The simultaneous administration of hydrocortisone appears to have no effect on this action of insulin and chlorpropamide.

Glutamic pyruvic transaminase activity augmented by the administration of hydrocortisone alone continued to be markedly increased despite the simultaneous administration of insulin and chlorpropamide.

Food restriction increased the activity of both transaminases. Under these conditions neither insulin nor chlorpropamide could be shown to exert any effect on GOT activity despite the further augmentation of GPT activity by hydrocortisone.

Further study is necessary to determine whether these changes in transaminase activity induced by these drugs are secondary in nature or whether they represent direct hormonal control.

### Acknowledgment

We acknowledge the valuable technical assistance we received from Carmine J. Caruso in the experiments reported in this paper.

### References

1. ROSEN, R., R. ROBERTS, L. E. BUDVICK & C. A. NICHOL. 1958. An enzymatic basis for the gluconeogenic action of cortisone. *Science* **127**: 287.
2. GAYOSTO, F. A., P. ILERI & A. BRUSCA. 1957. Increased transaminase activity in the liver after administration of cortisone. *Biochim et Biophys Acta* **11**: 250.
3. WROBLEWSKI, F. & J. S. LADLE. 1956. Serum glutamic pyruvic transaminase in cardiac and hepatic disease. *Proc Soc Exptl Biol Med* **91**: 569.
4. KARMEN, A. 1955. A note on the spectrophotometric assay of glutamic-oxaloacetic transaminase in human blood serum. *J Clin Invest* **34**: 131.
5. HILL, B. 1956. Some properties of serum lactic dehydrogenase. *Cancer Research* **16**: 460.
6. SASS, M. & P. W. SPEAR. 1958. Whole blood transaminase levels in anemia. *J Lab Clin Med* **51**: 926.
7. SASS, M. & G. MURPHY. 1958. The effect of isonicotinic acid hydrazide and vitamin B<sub>6</sub> on glutamic-oxaloacetic transaminase levels in whole blood. *Am J Clin Nutrition* **8**: 424.
8. STADIE, W. C. 1955. Current views on the mechanisms of insulin action. *Am J Med* **19**: 257.
9. HASTINGS, A. B., A. R. REYNOLD & J. C. T. TENG. 1955. Effect of ions and hormones on carbohydrate metabolism. *Recent Progr Hormone Research* **11**: 393.
10. BEATON, G. H. 1957. Alanine-glutamic transaminase and protein metabolism. *Arch Biochem Biophys* **70**: 289.
11. FERRARI, V. & L. T. TENCONI. 1955. Variations in some enzyme activities in the liver during gluconeogenesis due to prednisone. *Acta Vitaminol* **10**: 99.

- 12 LISCHER A M & C D KOCHALIAN 1954 Effect of cortisone acetate on aspartic glutamic transaminase activity of mouse tissue Proc Soc Exptl Biol Med 85 339
- 13 BRIN M & R W McREE 1956 Effects of X irradiation, nitrogen mustard, fasting, cortisone and adrenalectomy on GPT activity in the rat Arch Biochem Biophys 31 354
- 14 COPENHAVER J H E G SHIPLEY & R L MEYER 1951 Enzymes in the tissues of alloxan diabetic rats Arch Biochem Biophys 34 360
- 15 BOCH S J & E G HOLMES 1937 Effect of insulin on carbohydrate formation in liver Biochem J 31 89
- 16 JENSEN H & J L GRAY 1951 The influence of hormones on the amino acid dehydrogenase systems of the liver and kidney Ann N Y Acad Sci 54(4) 619
- 17 STADIE W C F D W LUKENS & J A ZAPP JR 1940 Effect of insulin upon urea formation, carbohydrate synthesis and respiration of livers of normal and diabetic animals J Biol Chem 132 393
- 18 COHEN P P & G L HERBUIIS 1941 Transamination in tumors, fetal tissues and regenerating liver Cancer Research 1 620
- 19 REICANT L & G L FISCHER 1957 Studies on the mechanism of tolbutamide hypoglycemia in animal and human subjects Ann N Y Acad Sci 71(1) 62
- 20 KNOX W E V H AUEBACH & E C C LIN 1956 Enzymatic and metabolic adaptations in animals Physiol Revs 36 164
- 21 ZICHLEWSKI A C & O H GAEBLER 1957 Changes in the activity of transaminases and L-glutamic acid dehydrogenase induced by growth hormone Arch Biochem Biophys 66 463

# SOME EFFECTS OF TOLBUTAMIDE AND CHLORPROPAMIDE IN VITRO\*

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## Introduction

Although a detailed discussion of available information concerning the site of action of the hypoglycemic sulfonylureas is beyond the scope of this paper, it is at the present time

impossible to consider well documented the position that the acute administration of the arylsulfonylureas leads to a decrease in the pancreatic insulin content<sup>1</sup> and to an increase in the insulinlike activity of pancreatic vein blood.<sup>2,3</sup> Increased circulating insulinlike activity, however, has not as yet been established in man.<sup>4,5</sup> Whether the same sequence of events holds true during long-term, chronic administration of these compounds is as yet unknown, as is the relative physiological importance of the postulated increased insulin secretion—either acutely or during long term administration. We also consider as well established the fact that the hypoglycemic effect of these compounds is

mildly stated—disputed.

The group for which I am speaking, associated with George W. Thorn's Metabolic Unit at Harvard Medical School and the Peter Bent Brigham Hospital, was impressed early by the existence of significant differences in the metabolic states induced by insulin and the sulfonylureas, respectively, even when the severity of the induced hypoglycemia was of similar magnitude and achieved in a similar period of time.<sup>6,7</sup> If one tends to accept the insulin stimulation theory of sulfonylurea action as the major site of action of these substances, this paradox may be resolved by endowing endogenous insulin secreted into the portal vein with special, previously unknown properties.<sup>8</sup> If, on the other hand, one is not particularly impressed by the evidence gathered for the over all physiological importance of the insulin stimulation theory of sulfonylurea action, one is led logically to give prominence to the extrapancreatic effects of the compounds. Our group still favors the latter interpretation, but I stress the fact that the following observations are not presented as additional evidence for or against the physiological importance of sulfonylurea induced insulin secretion. Since heated discussion on this

\* Research reported in this paper was supported by grants from Chas. Pfizer & Co., Inc. Brooklyn, N. Y. and The Upjohn Company, Kalamazoo, Mich.

† Recipient of a Research Fellowship from the Swiss Academy of Medical Sciences, Basel, Switzerland.

‡ Recipient of a Research Fellowship from the Dazian Foundation for Medical Research, New York, N. Y.

point seems rather fruitless at present, it is hoped that the effects shown will contribute to the over all pharmacodynamic description of these interesting agents. Indeed it may be permissible to expect that careful analysis of the extrapancreatic effects of the sulfonylureas, whatever their physiological significance may lead as well to the description of a mechanism for the sulfonylurea induced release of insulin.\*

### Material and Methods

We are here concerned exclusively with the effects of tolbutamide and chlorpropamide on ketogenesis by liver slices from rats fasted 48 hours, and with the drugs' effects on glucose metabolism by adipose tissue from fed normal rats. The methodology employed has been described previously in some detail<sup>10, 11</sup>. Alloxan diabetes was induced by the intravenous injection of 40 mg/kg alloxan following a 48 hour fast. Random blood glucose values exceeding 350 mg per cent within 2 or more weeks following alloxan injection were considered as indicative of the presence of diabetes. Total pancreatectomies were performed according to Scow<sup>12</sup> and only those animals exhibiting both hyperglycemia and ketosis 36 hours following pancreatectomy were used.

### Results

Boris Boshell of our laboratory has reported<sup>10</sup> that tolbutamide in concentrations of 1.0 mg/ml and 0.2 mg/ml significantly inhibits the formation of ketones by rat liver slices *in vitro*. The previously reported data are shown in TABLE 1. It was considered of particular significance that this

TABLE 1\*  
EFFECT OF TOLBUTAMIDE UPON KETOGENESIS BY LIVER SLICES FROM NORMAL RATS FASTED 48 HOURS

Addition to medium	No. of observations	Ketogenesis <i>in vitro</i>	
		Micrograms acetone equivalent/gm wet liver/90 min	Control (percentages)
No addition	7	610 ± 63†	100
Insulin 0.1 U/ml	7	590	97 ± 4†
Tolbutamide 1.0 mg/ml	7	226	38 ± 4
No addition	8	539 ± 100	100
Tolbutamide 0.2 mg/ml	8	376	70 ± 6

\* Data from Boshell *et al*<sup>10</sup>. Incubations were carried out in Krebs bicarbonate buffer modified by replacing some  $\text{Na}^+$  by  $\text{K}^+$  to final concentrations of  $\text{Na}^+ = 83$  and  $\text{K}^+ = 60$  ml/l.

† Mean ± standard error of the mean. || Once all experiments were paired experiments, the significance of the tolbutamide effect is calculated on the basis of the figures representing percentage of the control value.

tolbutamide effect could be demonstrated at a concentration (0.2 mg/ml or 20 mg per cent) quite frequently encountered in plasma or serum of tolbutamide treated patients and that the addition of tolbutamide at concentrations was ineffective in the presence of insulin mediation.

These observations have since been extended to include a greater range of concentrations and the comparison of chlorpropamide effects with the effects of tolbutamide. As shown in FIGURE 1, tolbutamide and chlorpropamide were approximately equally effective in depressing *in vitro* ketogenesis by liver slices from rats fasted 48 hours, although the data do not exclude

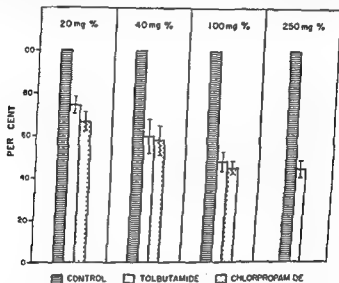


FIGURE 1. Effect of tolbutamide and chlorpropamide on ketogenesis by liver slices from rats fasted 48 hours. Top figures refer to the concentrations of drugs used expressed as milligrams per 100 ml. Incubations were carried out in a Krebs bicarbonate buffer modified by adjusting the concentrations of  $K^+$  to 60 and  $Na^+$  to 83 mEq/l.

the possibility that a differential potency of the two substances might have been detected at lower concentrations (for example, 10 mg per cent). The absence of a striking potency difference with regard to ketogenesis *in vitro* thus tentatively supports the view that *in vivo* potency differences of the 2 compounds are more likely to be related to differences in the metabolic fate of the drugs rather than to differences in their intrinsic metabolic activities.

FIGURE 2 further demonstrates the effects of tolbutamide and chlorpropamide upon hepatic ketogenesis as a function of time. It is evident that the effect appears early and persists throughout the period of incubation. The figure also demonstrates that the over-all drug induced inhibition is really greater than that previously estimated, since a major portion of the ketones present in the incubation medium (at the end of the incubation in the presence of tolbutamide and chlorpropamide) represents ketones

Whereas correlation between hypoglycemic activity *in vivo* and antiketogenic activity *in vitro* is at least consistent with the possibility of a common mechanism of action, further studies carried out in liver slices obtained from animals in which experimental diabetes had been induced by alloxan injection or by pancreatectomy failed to support this conclusion. As shown in FIGURE 3, tolbutamide effectively suppressed hepatic ketogenesis in the

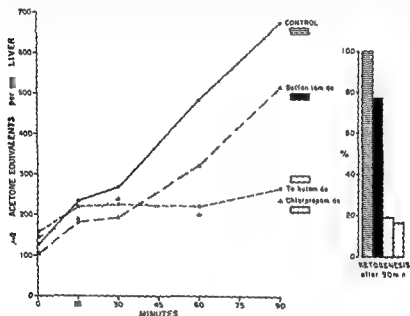


FIGURE 2 Effect of tolbutamide (100 mg per cent), chlorpropamide (100 mg per cent), and sulfanilamide (100 mg per cent) on ketogenesis by liver slices from rats fasted 48 hours. Each curve represents the mean of 2 experiments.

one group of alloxan-diabetic rats.

as it has been reported that tolbutamide

butamide for several days and his collaborators found that ketogenesis and relatively

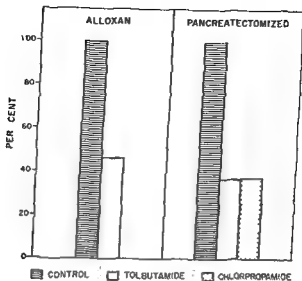


FIGURE 3 Effect of tolbutamide and chlorpropamide (100 mg per cent) on ketogenesis by liver slices from alloxan diabetic and pancreatectomized rats. Shown are the means of four (alloxan) and six (pancreatectomized) experiments.

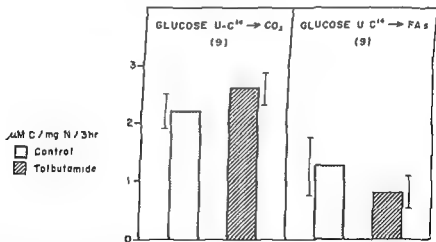


FIGURE 4 Effects of tolbutamide (0.8 mg/ml) upon rat adipose tissue *in vitro*. The standard errors shown do not take into account the paired nature of the data. The number of experiments is shown in parentheses. FA represents fatty acids.

$$\begin{aligned}\text{Tolbutamide effect } \text{CO}_2 &= +0.34 \pm 0.16 \\ \text{Fatty acids} &= -0.46 \pm 0.25\end{aligned}$$

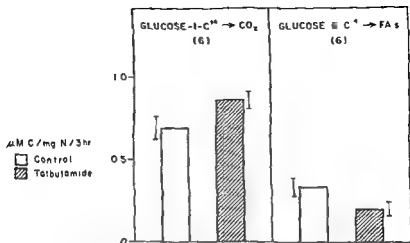


FIGURE 5 Effects of tolbutamide (0.8 mg/ml) upon rat adipose tissue *in vitro*. The standard errors shown do not take into account the paired nature of the data. The number of experiments is shown in parentheses. FA represents fatty acids.

Tolbutamide effect: CO<sub>2</sub> = +0.17 ± 0.07  
Fatty acids = -0.13 ± 0.05

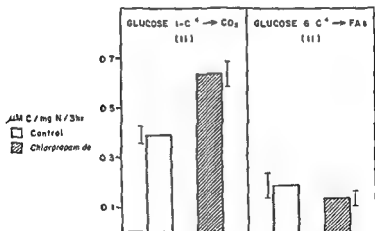


FIGURE 6 Effects of chlorpropamide (0.8 mg/ml) upon rat adipose tissue *in vitro*. The standard errors do not take into account the paired nature of the data. The number of experiments is shown in parentheses. FA represents fatty acids.

Chlorpropamide effect: CO<sub>2</sub> = +0.21 ± 0.03  
Fatty acids = -0.05 ± 0.07



acute administration of tolbutamide. Recently we investigated the effects of tolbutamide upon the metabolism of glucose by rat adipose tissue and observed (FIGURE 4) that the presence of tolbutamide (0.8 mg/ml) resulted in increased  $\text{CO}_2$  production from uniformly labeled glucose but in decreased lipogenesis from the same substrate. This combination of effects seemed rather unusual since as a rule increased glucose oxidation is also accompanied by increased synthesis of fatty acids from glucose carbon 1, 12, 13. When the observations were repeated with glucose 1- $\text{C}^{14}$  and glucose 6- $\text{C}^{14}$  as substrates (FIGURE 5) it appeared that the major contributor to the increased  $\text{CO}_2$  production from glucose was carbon 1 suggesting stimulation of the phosphogluconate oxidative pathway<sup>17</sup> while lipogenesis again was impaired. Finally FIGURE 6 demonstrates a clear cut stimulation of the oxidation of carbon 1 of glucose to  $\text{CO}_2$  in the presence of chlorpropamide (0.8 mg/ml) without concurrent stimulation of lipogenesis and indeed with a small inhibition of lipogenesis from glucose 6- $\text{C}^{14}$ .

### Discussion

The observations described simply add two further extrapancreatic effects of the arylsulfonylureas to those already reported on the rat hemidiaphragm<sup>18</sup> on a number of hepatic enzymes related to glycolysis or gluconeogenesis<sup>20, 21</sup> on epinephrine induced glycogenolysis<sup>22</sup> on hepatic transaminase activity<sup>23</sup> and on a number of  $\text{DPN}^+$  or  $\text{TPN}^+$ -dependent reactions.<sup>8, 9, 10, 11, 12, 13, 14, 15, 16</sup> The compounds described produced the following effects: (1) decreased  $\text{CO}_2$  production or increased fatty acid synthesis from acetate associated with decreased ketogenesis in the presence of acetate 1- $\text{C}^{14}$  as the substrate. Neither the synthesis of cholesterol nor the activity of the reactions leading to the production of acetoacetate from acetoacetyl coenzyme A however has as yet been measured under these conditions. It may be of some interest to point out that the observations relating to glucose oxidation and to lipogenesis from glucose by adipose tissue are in accord with the only attempt as yet made to find a common denominator for most or many effects of the arylsulfonylureas on enzyme systems. Wallenfels *et al.*<sup>8</sup> pointed out that the enzymatic reactions affected by these compounds are frequently dependent upon pyridine nucleotides as cofactors. Indeed they have even suggested a hypothetical mechanism of insulin release on this basis. The combination of increased  $\text{CO}_2$  production from carbon 1 of glucose with decreased fatty acid synthesis is compatible with the presence of some interference in the transfer of hydrogen from glucose 6 phosphate to fatty acids by way of reduced  $\text{TPN}^+$ . A similar mechanism has been postulated by Cahill and his collaborators<sup>24</sup> for the effects of methylene blue and certain other hydrogen acceptors in liver slices.

### Summary

Two extrapancreatic metabolic effects of tolbutamide and chlorpropamide have been described. First both substances have been shown to inhibit

animals. The mechanism by which this antiketogenesis is brought about is unknown. Second both substances affect glucose oxidation and lipogenesis from glucose by surviving rat adipose tissue in a manner suggesting interference with the proper transfer of hydrogen from glucose to the fatty acid chain perhaps at the level of the transferring pyridine nucleotides. The second observation is in accord with the generalization proposed by Wallenfels for a possible mechanism of action of the arylsulfonylureas upon tissues. The relevance of the two effects described to the hypoglycemic action of the arylsulfonylureas in man is unknown.

### References

1. PFEIFFER E F H, STEIGERWALD W, SANDRITTER A, BANDER A, MAGER U, BECKER & K. REIENE. 1957. Vergleichende Untersuchungen von Morphologie und Hormongehalt des Halberpankreas nach Sulfonilharntstoffen (D860). Deut. Med. Wochschr. 82: 1568.
2. LOUBATIERES A. 1955. L'utilisation de certaines substances sulfamidées dans le traitement du diabète sucré expérimental. recherches personnelles 1942-46. Presse méd. 63: 1701.
3. POZZA G, G. GALASSINO & P. P. FOI. 1956. Insulin secretion following carbutam de injection in normal dogs. Proc. Soc. Exptl. Biol. Med. 91: 539.
4. PFEIFFER E F *et al*. 1958. Proc. 3rd Congr. Intern. Diabetes Federation. Dusseldorf, Germany.
5. RENOLD A E, D. B. MARTIN, B. H. BOSHELL & G. W. THORN. 1957. Studies on the site of action of the arylsulfonylureas in man. II. Ann. N. Y. Acad. Sci. 71(1): 71.
6. PROLT T F, G. W. SCOTT, J. A. WEAVER & S. P. ASPER, JR. 1958. Studies of the Metabolism of insulin. Bull. Johns Hopkins Hosp. 102: 46.
7. RENOLD A E, A. I. WINEGRAD, I. R. FROESCH & G. W. THORN. 1956. Studies on the site of action of the arylsulfonylureas in man. Metabolism 5: 757.
8. MADISON L. B., COMBES H. H., UNGER N. & KAPLAN. 1959. The relationship between the mechanism of action of the sulfonylureas and the secretion of insulin into the portal circulation. Ann. N. Y. Acad. Sci. 74(3): 548.
9. WALLENFELS K. H. D. SUMM & W. CREUTZFELDT. 1957. Enzymatische Untersuchungen zum Wirkungsmechanismus der blutzuckersenkenden Medikamente. Deut. Med. Wochschr. 82: 1581.
10. BOSHELL B. R., G. R. ZAHND & A. F. RENOLD. 1959. An effect of tolbutam de on ketogenesis *in vivo* and *in vitro*. To be published.
11. WINEGRAD A. I. & A. F. RENOLD. 1958. Studies on rat adipose tissue *in vitro*. I. Effects of synthesis on the metabolism of glucose, pyruvate and acetate. J. Biol. Chem. 233: 267.
12. SCOW R. O. 1957. Total pancreatectomy in the rat: operation effects and postoperative care. Endocrinology 60: 359.
13. FROMM S. & H. C. PLATTNER. 1957. Influence d'un sulfamidé hypoglycémiant sur la synthèse des graisses chez la souris. Arch. Sci. Geneva 10: 261.
14. ASHMORE J. G. F., CARILL, JR. A. S., EARLE & S. ZOTT. 1958. Studies on the disposition of blood glucose. Diabetes 7: 1.
15. SIFFERSTEIN M. D. & V. M. FAGAN. 1957. Role of glycolysis in fatty acid and cholesterol synthesis in normal and diabetic rats. Science 126: 1012.
16. TEPPERMAN J. & H. M. TEPPERMAN. 1958. Effects of antecedent food intake pattern on hepatic lipogenesis. Am. J. Physiol. 193: 55.
17. WINEGRAD A. I. & A. F. RENOLD. 1958. Studies on rat adipose tissue *in vitro*.

II Effects of insulin on the metabolism of specifically labeled glucose J Biol Chem 233 272

- 18 PLETSCHER A & K I GEY 1957 Über die Wirkung Glukozersenkender Sulfonylharnstoffe auf das isolierte Rattenzwerchfell Experientia III 447
- 19 LUNDBAEC K K NIELSEN & O J RAFAELSEN 1958 Mode of action of oral antidiabetic compounds Lancet I 1036
- 20 CAHILL G F JR A B HASTINGS & J ASHMORE 1957 Effects of substituted sulfonylureas on rat diaphragm and liver tissue Diabetes 6 26
- 21 WEBER G & A CANTERO 1958 Effect of orinase on hepatic enzymes involved in glucose 6-phosphate utilization Metabolism 7 333
- 22 VAUGHAN M 1957 The effect of tolbutamide on glucose production by the liver *in vitro* Ann N Y Acad Sci 71(1) 112
- 23 BORNSTEIN J 1957 Inhibition of alanine transaminase by the hypoglycemic sulphonylurea derivatives Nature 179 534
- 24 CAHILL G F JR A B HASTINGS J ASHMORE & S ZOTTU 1958 Studies on carbohydrate metabolism in rat liver slices V Factors in the regulation of pathways of glucose metabolism J Biol Chem 230 125

## Part II Metabolic Studies

### INHIBITION OF THE DIABETOGENIC ACTION OF SOMATOTROPIN BY TOLBUTAMIDE\*

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The mechanism whereby the daily administration of diabetogenic extracts of the anterior pituitary gland or of purified growth hormone (somatotropin) to the dog results in the development of a temporary diabetic state (idiopathic hypophyseal diabetes) that is followed by a permanent diabetic state (metahypophyseal diabetes) is still unknown. A good deal of evidence can be marshalled in support of the hypothesis that somatotropin induces an increased peripheral need for insulin with a resultant increase in the production of insulin by the beta cells of the pancreas.<sup>1,2</sup> Accordingly, it has been proposed that when the rate of insulin utilization exceeds the rate at which it is manufactured the beta cells are stimulated excessively and undergo degranulation, hydropic degeneration and atrophy. Thus the idiopathic hypophyseal diabetes is attributed to a relative insufficiency in the capacity of the pancreas to meet the increased peripheral requirements for insulin, while the metahypophyseal diabetes that persists after the cessation of the somatotropin injections is attributed to the exhaustion, degeneration and destruction of the beta cells.<sup>1,3</sup>

The same sequence of morphologic changes that is observed in the islet tissue of the somatotropin treated dog<sup>2</sup> is found also during the development of diabetes in the partially depancreatized cat<sup>4</sup> and dog<sup>5</sup> and in the normal or partially depancreatized dog or cat which develops a metadiabetes after the administration of thyroid extracts,<sup>6</sup> anterior pituitary extracts,<sup>4,8,11,12</sup> adrenal corticosteroids<sup>13</sup> or glucose.<sup>14</sup> Degranulation and various degrees of hydropic degeneration of the beta cells are found also in animals with the idiadiabetes produced during the course of the administration of anterior pituitary extracts or somatotropin,<sup>2,11,12</sup> corticosteroids,<sup>12,15</sup> and large quantities of glucose.<sup>16,16</sup> Concomitant with the degranulation and hydropic degeneration is a reduction in the concentration of extractable insulin in the pancreas.<sup>7,12,21,22</sup>

In accord with the hypothesis that an excessive demand for insulin is a determinant in the production of metadiabetes are the observations that regimes that reduce the peripheral requirements for insulin may also reduce the severity of the idiadiabetes and prevent the development of the metadiabetes produced by various diabetogenic agents.<sup>23</sup> Fasting, undernutrition, a high fat diet, and treatment with insulin appear to reduce the require-

\* The work reported in this paper was supported in part by Grant A 1409 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

ments for insulin and thereby reduce the production of insulin by the pancreas<sup>21, 27</sup> These measures inhibit the effect of diabetogenic factors in varying degrees Thus the changes in the morphology of the islets of Langerhans and the metadiabetes that may occur in partially depancreatized animals are inhibited or corrected by undernutrition<sup>28</sup> and by the administration of insulin<sup>29</sup> Likewise the diabetogenic action of anterior pituitary preparations is diminished or inhibited by undernutrition high fat diets and the administration of insulin<sup>11, 14, 22, 30</sup>

Although it is generally agreed that the degranulation of the beta cells produced by diabetogenic agents is due to excessive stimulation of the production and or release of insulin by the beta cells the significance of the hydropic degeneration in the development of metadiabetes remains unknown The observations of Toreson<sup>31</sup> and of Duff and Toreson<sup>32</sup> established that the morphologic changes identified as 'hydropic degeneration' reflect the deposition of large amounts of glycogen in the beta cells These studies were extended by Lazarus *et al.*<sup>7, 33</sup> who employed a variety of diabetogenic agents and concluded that hydropic degeneration is not due to exhaustion of the beta cells but is a facet of the increased glycogen deposition in cells such as occurs in the kidney and heart of the diabetic animal These studies however do not negate the hypothesis that excessive stimulation of the beta cells is responsible for their exhaustion and atrophy

Irrespective of the precise mechanism whereby metadiabetes is produced by somatotropin it is now quite evident that stimulation of the beta cells and the release of insulin into the circulation is an early effect of its action<sup>1, 3</sup> Thus the administration of purified somatotropin (2 to 3.5 mg/kg/day) to dogs for 6 to 8 days results in an extensive degranulation of the beta cells and a reduction in the concentration of extractable insulin in the pancreas this may occur even before the development of hyperglycemia<sup>7</sup> The extent and the rapidity with which the degranulation occurs is similar to that produced by a number of agents that do not produce a permanent diabetes<sup>22</sup>

lycemic action of isopropylthio

the action of this and related

sulfonamide derivatives is dependent upon the presence of more than one tenth to one fifth of the pancreas Since the introduction of carbutamide and tolbutamide as orally effective hypoglycemic agents it has been firmly established that their hypoglycemic action is dependent upon the presence of islet tissue capable of producing insulin<sup>4</sup> Although it is quite probable that a number of mechanisms are involved in the action of these agents there is ample evidence that one of these mechanisms is the stimulation of the beta cells and the release of insulin from pancreatic stores<sup>23, 26</sup>

Of particular interest are the observations that the administration of

in the pancreas 11111

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physiological effects that are somewhat similar to those produced during the early stages of the administration of somatotropin as suggested by the dimin

ished glucose tolerance that occurs in rats and dogs treated with carbutamide for a prolonged period.<sup>27</sup> In accord also is the demonstration that the usual hypoglycemic phase of the response to the injection of alloxan does not occur in rats given large doses of tolbutamide for 3 or more days.<sup>41</sup>

Although some of the early effects of somatotropin appear to be similar to those produced by the sulfonylureas the mechanisms involved appear to be quite different. Whereas the excessive stimulation of the beta cells that occurs in the dog treated with somatotropin is probably secondary to an increase in the peripheral requirements for insulin that resulting from the administration of the sulfonylureas is probably due to a direct effect on the islets. Thus the insulin requirements of the completely depancreatized dog are increased by somatotropin<sup>1,2,3</sup> and not by the sulfonylureas.<sup>41,42</sup> Nevertheless the similarity in the morphologic changes produced by these agents prompted a study on the effect of somatotropin on the acute hypoglycemic action of a single injection of tolbutamide and on the effect of the daily oral administration of tolbutamide on the diabetogenic action of somatotropin.

A purified preparation of bovine somatotropin\* was given to mile dogs weighing from 10 to 17 kg. The hormone was injected intramuscularly in a daily dosage that varied from 3 to 10 mg/kg. The animals were given a constant daily diet and any food not eaten by 4:30 P.M. was removed and weighed. Between 8 and 9 every morning the animals were weighed, venous blood samples were taken and somatotropin was injected. Following these procedures the dogs were fed. In some instances blood samples for sugar determination were taken at intervals throughout the day. The daily urine excretion was collected quantitatively. The concentration of sugar in the blood and urine was determined by Nelson's method.<sup>43</sup>

One group of somatotropin treated animals was given a single intravenous injection of tolbutamide† (25 mg/kg) at various intervals before, during and after the course of injections of somatotropin. Samples of blood were drawn from the fasted animals at intervals before and for 3 hours after the injection of the tolbutamide. On the day of the test the administration of somatotropin was withheld until the test was completed. A second group of dogs was similarly treated with somatotropin and in addition, was given a single oral dosage of tolbutamide (50 to 100 mg/kg/day).

The administration of somatotropin (3 to 8 mg/kg/day) for from 4 to 6 days results in a change in the character of the hypoglycemic response to the intravenous injection of a standard dose of tolbutamide. Whereas the normal dog responds to tolbutamide within 30 min with a precipitous decrease in the blood sugar concentration the animal treated with somatotropin for 4 to 6 days responds with a relatively slow progressive decrease. The inhibition of the first phase of the normal acute response to tolbutamide may occur even before the hormone has produced a significant hyperglycemia.<sup>40</sup>

\* The somatotropin used in this study is a growth hormone preparation provided by the Endocrinology Study Section, National Institutes of Health.

† We are indebted to The Upjohn Company, Kalamazoo, Mich. for supplies of tolbutamide (Orinase).

the period of treatment. The response of the dogs that were given both somatotropin and tolbutamide, however, differed from the above in that only a relatively moderate increase in the blood sugar concentration occurred. The difference between the responses of the two groups of animals is statistically highly significant ( $p < 0.001$ ).

Illustrative of the responses of individual animals comprising each group of dogs are the data depicted in FIGURES 3 and 4. By the fifth day dog 3

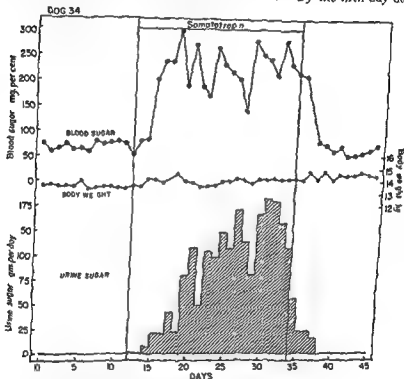


FIGURE 3 The effect of tolbutamide on the diabetogenic action of somatotropin. Dog 34 was given intramuscular injection of somatotropin (6 mg/kg/day) throughout the designated period.

(FIGURE 3) developed a significant hyperglycemia and glycosuria that persisted throughout the period during which somatotropin was injected. Dog 20 (FIGURE 4) however received tolbutamide and developed a hyper-

sensitivity to the diabetogenic action of the somatotropin. Six dogs were designed in which each animal served as its own control. Six dogs were given 2 courses of injections with somatotropin, 3 received tolbutamide orally during the first course, and 3 were given the tolbutamide during the second course of injections. Illustrative of the first group of dogs are the

data on dog 31 illustrated in FIGURE 5. In this instance the tolbutamide was not started until a definite hyperglycemia and glycosuria were present. Although the blood sugar remained above the normal concentration it was significantly lower than that produced in the same dog when it was not given any tolbutamide during the second course of injections which was started after an interval of 13 days.

That the relatively short interval between the 2 courses of somatotropin injections is not responsible for the more marked diabetogenic response to the second course of injections and for the development of metadiabetes in dog

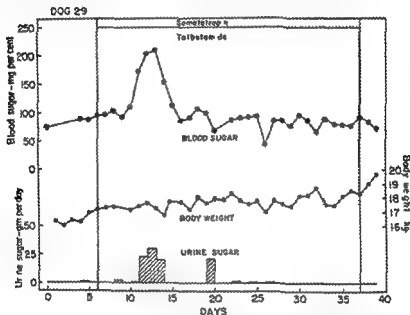


FIGURE 4 The effect of tolbutamide on the diabetogenic action of somatotropin. Dog 29 was given the same dosage of somatotropin (6 mg/kg/day) as dog 34 (see FIGURE 3) plus tolbutamide (50 mg/kg/day orally).

31 (FIGURE 5) is indicated by the data on dog 38 (FIGURE 6). This dog was given intramuscular injection of somatotropin (8 mg/kg/day) and after a significant hyperglycemia and glycosuria developed the animal was given tolbutamide (100 mg/kg) on the days indicated. The blood sugar fluctuated between normal and hyperglycemic levels and a moderate degree of glycosuria occurred. The second course was started 340 days after the first course of injections of somatotropin was stopped. It is quite apparent that in spite of the lengthy interval between the 2 courses of injections a much more marked hyperglycemia and glycosuria occurred during the second course and the animal developed a metadiabetes.

In complete agreement with the above are the data obtained in the experi-



ments in which the tolbutamide was given during the second course of injections of somatotropin. Typical of these are the data illustrated in FIGURE 7. Whereas a significant hyperglycemia and glycosuria developed during the course of injections of somatotropin, a marked inhibition of the diabetogenic response occurred during the second course, when tolbutamide was given daily by mouth. In no instance did a metahypophyseal diabetes develop after this second course of injections.

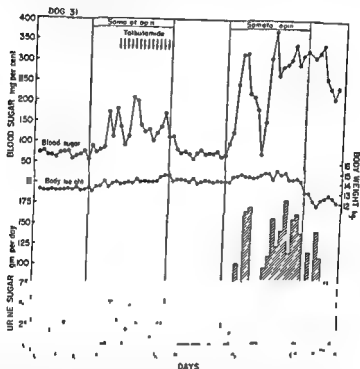


FIGURE 5 Effect of tolbutamide on the diabetogenic action of somatotropin. During the first course of intramuscular injections of somatotropin (5 mg/kg/day), tolbutamide (100 mg/kg) was given orally at designated days (arrows). A metahypophyseal diabetes followed the cessation of the second course of injections of somatotropin.

Illustrative of another experimental design that was used in this study are the data depicted in FIGURE 8. Five days before beginning the administration of somatotropin (5 mg/kg/day), dog 37 was started on an oral dose of tolbutamide (25 mg/kg/day). The tolbutamide was increased to 50 mg/kg/day on the tenth day after the first injection of somatotropin. During the interval a hyperglycemia developed gradually. Within 2 days after the tolbutamide dosage was increased, the blood sugar concentration began to fall and reached the pretreatment level by the fifth day. On the twenty-sixth day after the first injection of somatotropin (that is, 11 days after the blood sugar concentration reached the control level) the tolbutamide

was discontinued but the injection of somatotropin (5 mg/kg/day) was continued for another 27 days. The blood sugar remained unchanged throughout this period. When the dosage of somatotropin was increased to 10 mg/day there was no change in the fasting blood sugar concentration even though there was a higher concentration of glucose in the samples taken at 4 P.M. With the injection of somatotropin (15 mg/kg/day) the fasting blood sugar increased and remained elevated even when the dosage was

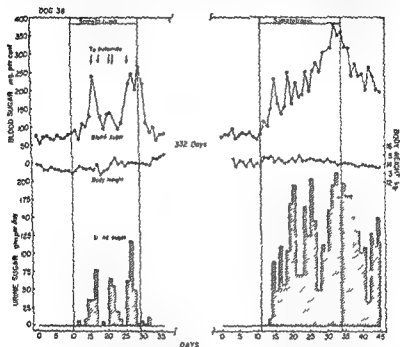


FIGURE 6. Effect of tolbutamide on the diabetogenic action of somatotropin. During the first course of intramuscular injections of somatotropin (8 mg/kg/day) the animal was given tolbutamide (100 mg/kg) orally at designated days (arrows). A metabolic hypoglycemia follows the cessation of the second course of injections with somatotropin

reduced to 10 mg/kg/day. With the cessation of somatotropin injections the blood sugar returned to normal levels.

The observations reported herein establish the fact that the administration of appropriate quantities of tolbutamide decreases or inhibits the diabetogenic response to the intramuscular injection of somatotropin. Such an effect could not be anticipated on the basis of the morphologic changes in the pancreas that follow the administration of each agent. As noted above, each agent stimulates the pancreas and induces degranulation and hydropic degeneration of the beta cells. If such stimulation and the resultant morphologic changes play a role in the subsequent destruction of the islet

tissue then the concomitant administration of both agents would be expected to enhance the rate of beta cell destruction and the development of metadiabetes. The validity of the concept stimulation of the pancreas, with a consequent exhaustion and degeneration of the beta cells.

It can be postulated that the tolbutamide induces a discharge of insulin by direct stimulation of the pancreas and that the resultant increase in circulating hormone nullifies the effects of an increase in peripheral requirements

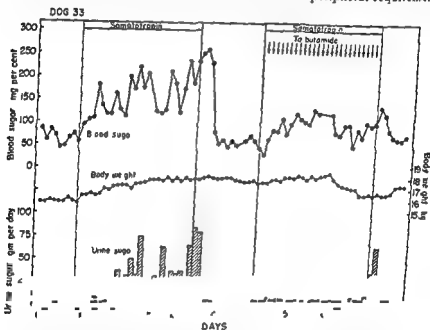


FIGURE 7 Effect of tolbutamide on the diabetogenic action of somatotropin. During the second course of intramuscular injections of somatotropin (5 mg/kg/day) tolbutamide (50 mg/kg/day) was given orally.

for insulin thereby obviating the diabetogenic action of somatotropin. The observations illustrated in FIGURE 1 however suggest that tolbutamide is ineffective in producing a further stimulation of the discharge of insulin after the first few days following the onset of somatotropin injections. Further blood samples taken at various intervals during the day from animals treated with both somatotropin and tolbutamide usually showed that the fasting blood sugar was the lowest of the day.

An alternative hypothesis is suggested by Anderson and Long's<sup>12</sup> observation that the perfused pancreas stops its secretion of insulin when a growth hormone preparation is added to the perfusate. Thus somatotropin may exert some direct action on the beta cells that inhibits their secretory activity. Accordingly, the effect of tolbutamide could be attributed to an action in the

beta cell that protects some intracellular mechanism which is vulnerable to the action of somatotropin. It is difficult, however, to postulate a mechanism whereby such a protective action could be effective for as long a period as that observed in dog 37. However the observation that pretreatment with tolbutamide results in an amelioration of the diabetes that is subsequently produced by the injection of alloxan<sup>41</sup> supports the possibility that tolbutamide exerts some protective action in the beta cells. Further clarification may come with similar and more extensive studies with other diabetogenic agents.

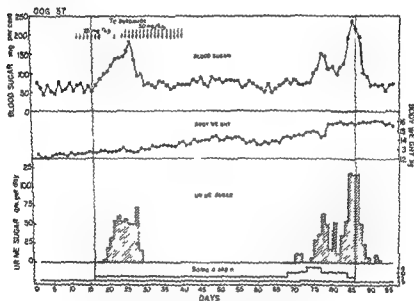


FIGURE 3 Effect of tolbutamide on the diabetogenic action of somatotropin (see text).

The data presented herein offer no new insight to the mechanism responsible for the diabetogenic action of somatotropin. They do suggest that the growth arrest type of diabetes mellitus is not due to the action of somatotropin. In contrast with the effect of tolbutamide on the somatotropin-treated dog, the diabetic child who is responsive to tolbutamide at the onset of the syndrome usually loses his responsiveness with the passage of time irrespective of whether he is treated with tolbutamide<sup>42</sup> or with insulin.<sup>43</sup> The patient with acromegaly, however, who develops diabetes should be responsive to tolbutamide until such time as the beta cells of his pancreas are destroyed. Accordingly it is probable that an idiopathic diabetes is responsible for the hyperglycemia of patients with acromegaly who have been found to respond to tolbutamide.<sup>43</sup> The prophylactic action of tolbutamide in such patients must await further study.

## Summary

The intravenous injection of a single dose of tolbutamide (25 mg/kg) to normal dogs results in a precipitous decrease in the blood sugar concentration within 30 min. Within a few days after the beginning of the daily intramuscular injection of somatotropin the injection of a similar dose of tolbutamide results in a gradual decrease in the blood sugar. Subsequently with the continued administration of somatotropin the hypoglycemic response to the injection of tolbutamide is completely inhibited.

The diabetogenic effect of the daily intramuscular injections of somatotropin is decreased or inhibited by the concomitant oral administration of tolbutamide.

## References

- 1 YOUNG F G 1953 The growth hormone and diabetes. *Recent Progr Hormone Research* 8: 471
- 2 CAMPBELL J 1955 Diabetogenic action of growth hormone. In *Hypophyseal Growth Hormone: Nature and Actions*. R W Smith, G H Gaebler and C A H Long, Eds. McGraw Hill, New York, N. Y.
- 3 RANDLE P J 1957 The growth hormone of the pituitary gland and metabolic processes. In *The Biological Action of Growth Substances*. Symposia Soc Exptl Biol 11: 183
- 4 RICHARDSON A C & F G YOUNG 1938 *Histology of diabetes in mice*. In *Diabetes Mellitus*. J. H. Little, Ed. McGraw-Hill, New York, N. Y.
- 5 RICHARDSON A C 1938 *Histology of diabetes in mice*. *J. Pathol. Bact.* 45: 177
- 6 RICHARDSON A C 1938 *Histology of diabetes in mice*. *J. Pathol. Bact.* 45: 177
- 7 CAMPBELL J H & HAUSSLER J S MOYER & I W F DAVIDSON 1953 Effects of growth hormone in dogs. *Endocrinology* 53: 131
- 8 HOMANS J 1914 Degeneration of the islands of Langerhans associated with experimental diabetes in the rat. *J. Med. Research* 30: 49
- 9 ALLAN F M 1922 Pathology of diabetes: hydropic degeneration of islands of Langerhans after partial pancreatectomy. *J. Metabolic Research* 1: 5
- 10 HOUSSEY H A 1944 *Diabetes Mellitus*. *Endocrinology* 35: 158
- 11 LUKENS F D W & F D W LUKENS 1948 *Diabetes Mellitus*. *Endocrinology* 35: 158
- 12 BEST C H & J CAMPBELL 1953 *Diabetes Mellitus*. *Endocrinology* 35: 158
- 13 HOUSSEY H A, L F HARTMAN & A F CARDEZA 1955 Metacorticotid diabetes in the dog. *Diabetes* 4: 146
- 14 DOHAN F C & F D W LUKENS 1948 Experimental diabetes produced by the administration of glucose. *Endocrinology* 42: 244
- 15 KOBERNICK S D & R H MORE 1950 Diabetic state with lysaemia and hydropic changes in the pancreas produced in rabbits with cortisone. *Proc Soc Exptl Biol Med* 74: 602
- 16 HAUSSLER F & A J RAMSAY 1953 Steroid diabetes in guinea pigs: effects of cortisone administration on blood and urinary glucose, nitrogen excretion, fat deposition and the islets of Langerhans. *Endocrinology* 53: 423
- 17 VOLK B W & S S LAZARUS 1958 The effect of various diabetogenic hormones on the structure of the rabbit pancreas. *Am J Pathol* 34: 121
- 18 WOERNER C A 1949 Studies of the islets of Langerhans after continuous intravenous injections of dextrose. *Anat Record* 72: 71

- 19 BARON S S & D STATE 1949 Effect of prolonged intravenous administration of dextrose on beta cells of islets of Langerhans *Am J Arch Pathol* 48 297
- 20 BROWN I M F C DOHAN L R FREEDMAN P DE MOOR & F D W LLAENS 1952 The effects of prolonged infusion of dogs pancreas with glucose *Endocrinology* 50 644
- 21 HAIST R F 1944 Factors affecting the insulin content of the pancreas *Physiol Revs* 24 409
- 22 WRENSHALL G A A BOGACH & R C RITCHIE 1952 Extractable insulin of the pancreas Correlation with pathological and clinical findings in diabetic and non diabetic cases *Diabetes* 1 87
- 23 HAIST R F J CAMPBELL & C H BEST 1940 The prevention of diabetes *New Engl J Med* 223 607
- 24 BEST C H R F HAIST & J H RIBOUT 1939 Diet and the insulin content of the pancreas *J Physiol* 87 107
- 25 TEJNING S 1947 Dietary factors and quantitative morphology of the islets of Langerhans *Acta Med Scand Suppl* 198 1
- 26 HAIST R E M EVANS M KIVACH I L BRYAN & M A ISHWORTH 1949 Factors affecting the volume of the islands of Langerhans *Proc Am Diabetes Assoc* 9 31
- 27 BEST C H & R F HAIST 1941 The effect of insulin administration on the insulin content of the pancreas *J Physiol* 100 142
- 28 ALLEN F M 1922 Pathology of diabetes IV Role of hyperglycemia in production of hydropic degeneration of islands of Langerhans *J Metabolic Research* 1 75
- 29 COPE L F F & A J BARCLAY 1923 The restoration of hydropically degenerated cells of the pancreatic islands in dogs under insulin treatment *J Metabolic Research* 4 440
- 30 HOUSSAY B A 1937 Diabetes as a disturbance of endocrine regulation *Am J Med Sci* 193 581
- 31 TORGON W L 1951 Glycogen infiltration (so called hydropic degeneration) in the pancreas in human and experimental diabetes mellitus *Am J Pathol* 27 327
- 32 DUFF G L & W F TORGON 1951 Prevention and reversal despite hyperglycemia of glycogen infiltration (hydropic degeneration) in the pancreas of alloxan diabetes in the rat *Endocrinology* 48 293
- 33 LAZARUS S S & M W VOLA 1953 Pathogenesis of hydropic degeneration (glycogen deposition) in the diabetic pancreas *Diabetes* 7 15
- 34 JANDON M J CHAPTEL A VRAEL & J SCHAAF 1942 Accidents hypoglycémiques graves par un sulfamide isothiodiazol (le Vh 57 ou 2254 RP) *Montpellier Méd* 21 441
- 35 LOUBATIERES J 1957 The hypoglycemic sulfonamides history and development of the problem from 1942 to 1955 *Ann N Y Acad Sci* 71(1) 4
- 36 THE EFFECTS OF SULFONYLUREAS AND RELATED COMPOUNDS IN EXPERIMENTAL AND CLINICAL DIABETES K LEVINE Ed 1957 *Ann N Y Acad Sci* 71(1) 1
- 37 VON HOLT C J KRAFFT & L VON HOLT 1956 Wirkung von N-sulfanylnitrosobutylcarbamid auf kohlenhydratstoffwechsel und endokrines system *Schwed med Wochschr* 86 1129
- 38 KRETSCHMAYR W & H FINTEN 1956 Blutzucker und histologische Veränderungen nach D 360 bei normalen Kanarienvögeln *Deut med Wochschr* 81 892
- 39 VOLK B W M G GOLDMAYR S WEISSENFELD & S S LAZARUS 1957 Functional and histological studies concerning the action of sulfonylureas *Ann N Y Acad Sci* 71(1) 141
- 40 VOLA M W & S S LAZARUS 1953 Pathogenesis of Ornithine induced beta cell degeneration in *Diabetes* 7 125
- 41 KIVACH J E & G W SEARLE 1958 Tolbutamide and the onset of alloxan diabetes in rats *Diabetes* 7 343
- 42 RUOT M A 1957 Effect of tolbutamide on the insulin content of the dog pancreas *Diabetes* 6 12
- 43 CAMPBELL J J S MINTOFF H R HALLER & J W F DAVENPORT 1953 Effects of growth hormone in diabetic dogs *Endocrinology* 53 549

- 44 CAMPBELL J 1956 The action of BZ 55 on dogs II Observation on depan-  
creatized and metahypophyseal diabetic dogs Can Med Assoc J 71 962
- 45 RICKETS H T H L WILDBERGER & H SCHMID 1957 Long term studies of the  
sulfonylureas in totally depancreatized dogs Ann N Y Acad Sci 71(1) 10
- 46 LOUBATIÈRES A 1957 The mechanism of action of the hypoglycemic sulfonamides:  
a concept based on investigations in animals and in human beings Ann N Y  
Acad Sci 71(1) 192
- 47 HOUSSAY H A J C PENHOS E URGOTTI N TEODOSIO J APPELBAUM & J BOWEN  
1957 The role of insulin in the action of the hypoglycemic sulfonyl compounds  
Ann N Y Acad Sci 71(1) 25
- 48 CABEN R & L CARBO 1957 The potentiation of exogenous insulin by tolbutamide  
in depancreatized dogs J Clin Invest 31 1546
- 49 NELSON N 1944 A photometric adaptation of the Somogyi method for the deter-  
mination of glucose J Biol Chem 153 375
- 50 MIRSKY I A G PERISUTTI & S GITELSON 1957 The role of insulinase in the  
hypoglycemic response to sulfonylureas Ann N Y Acad Sci 71(1) 103
- 51 MIRSKY I A D DIENGOTT & H DOLGER 1956 The relation of various variables  
to the hypoglycemic action of 1-butyl-3-p-tolylsulfonylurea in patients with diabetes  
mellitus Metabolism 5 875
- 52 " " " " " " " " " " " "
- 53 .
- 54
- 55  
of tolbutamide on the diabetes of acromegaly and on the blood sugar in patients  
with altered endocrine states Ann N Y Acad Sci 71(1) 215

## DEGRADATION OF INSULIN- $I^{131}$ AND GLUCAGON- $I^{131}$ AND FACTORS INFLUENCING IT\*

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As recently reported<sup>1,2</sup> insulin is rapidly degraded in the body and apparently before some of the hormone has exerted its action. A better understanding of the factors influencing the degradation of this hormone conceivably could be of assistance in controlling diabetes. In this paper are reported results of some of our studies of the capacity of slices and homogenates of most of the body tissues to degrade insulin  $I^{131}$ . Also are reported the influence on this hormone's degradation of infection, trauma, and many factors that presumably influence the amount of insulin secretion, including starvation, phlorrhization, alloxan diabetes, prednisolone, insulin hypophysectomy, adrenalectomy or diets high in carbohydrate, fat, or protein.

Since glucagon also exerts a powerful influence on carbohydrate distribution and, indeed, may cause diabetes,<sup>3</sup> certain phases of the metabolism of this hormone are reported.

### METHODS

For these studies insulin and glucagon labeled with  $I^{131}$  were used. Since we have reported many previous observations<sup>1-6</sup> with these labeled compounds, details of the techniques employed are discussed in this paper only very briefly. However the following points need particular emphasis. Great caution must be exercised in the iodination of the hormones because heavy iodination and/or radiation will abolish their chief biological activity and alter their chemical and physical behavior. With the iodination techniques employed in our preparations full biological activity can be preserved. However, there still tends to be some variation from one batch to the next in some of the chemical properties, necessitating the performance of a sufficient number of control experiments with each batch. For the minimization of the adsorption of the hormones to glassware and for completeness in the precipitation of the hormone it is desirable to have at least several milligrams of protein in the container, we commonly used normal plasma. In order to work with the most satisfactory ratios of enzyme to substrate we routinely added the corresponding nonlabeled hormone.

All insulin  $I^{131}$  used was purchased from the Abbott Laboratories, North Chicago, Ill., and was dialyzed at 4° C. for several hours immediately before use. An insulin-degrading enzyme preparation was used and consisted either of tissue homogenate (liver in most instances) or of the dried powder remaining after special treatment of beef liver homogenate with acetone and ether.

\* The work reported in this paper was supported in part by Grants A 575 and A 2436 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md., and in part by the Warren Orr Research Fund and The Diabetes Teaching and Research Foundation, Seattle, Wash.



The portion of 4 mg of the extracted liver that was soluble in 0.5 ml of buffer or a specific amount of tissue homogenate was placed in a beaker. Either 0.067 M phosphate or, more often, 0.1 M tris (hydroxymethyl)amino methane (Tris), containing  $5 \times 10^{-2}$  M Versene was used as a buffer. To the enzyme preparation was added 0.4  $\mu$ g of insulin  $I^{125}$  and 0.1 mg of amorphous insulin, in this system the quantity of enzyme is the limiting factor in the rate of the reaction. The total 2 ml mixture, with the pH adjusted to 7.4, was placed in a Dubnoff metabolic shaker at 37° C for 5 min. Fifteen seconds before the end of the incubation 1 ml of 2 per cent of plasma in water was added to ensure complete precipitation of insulin, at the end of the incubation period 3 ml of 20 per cent trichloroacetic acid (TCA) was added. After standing for 30 min the mixture was centrifuged. The solid residue was washed 2 to 5 times with 10 per cent trichloroacetic acid, and the washings were added to the supernatant. Each sample was run in triplicate.

The radioactivity in each specimen was measured by a well type gamma counter. The precipitate was dissolved in 30 per cent KOH and brought to a volume equal to that of the combined supernatants. A calculation was then made of the percentage of the total radioactivity found in the supernatant fractions; this fraction was regarded as an indicator of the amount of degraded hormone. At least the following 2 control studies were conducted simultaneously with each experiment: (1) 1 ml of buffer plus 1 ml of insulin solution, and (2) 0.5 ml of buffer plus 0.5 ml of enzyme preparation plus 1 ml of insulin solution. The exceptions to the foregoing technique are described where indicated.

All rats were male and, except as noted later, were of the Sprague Dawley strain.

#### INSULIN $I^{125}$ DEGRADATION BY HOMOGENATES OF DIFFERENT TISSUE

Nonfasted rats approximately 300 gm, male and female, approximately 30 days old, gastrocnemius muscle, pH 7.5 and homogenized. From 1 to 15 per cent concentrations of the homogenates were incubated with insulin  $I^{125}$  (0.5  $\mu$ Ci, 1  $\mu$ g) and 13  $\mu$ g of amorphous insulin. The amount of degradation was determined as described under METHODS.

As seen in TABLE 1, there was an increase in the degradation of insulin  $I^{125}$ , with an increase in the concentration of homogenate used. In concentrations from 1 to 7.5 per cent the effect was essentially linear, in spite of the fact that the experiments were not conducted simultaneously or with the same batch of insulin  $I^{125}$ . Liver and kidney were much more active than gastrocnemius muscle.

Our studies were extended to determine to what extent other tissue homogenates would degrade insulin  $I^{125}$ .

0.5 ml was used in each vessel.

The results are shown in TABLE 2. Since 6 different experiments were con-

TABLE 1  
INSULIN  $I^{131}$  DEGRADATION BY DIFFERENT CONCENTRATIONS OF  
TISSUE HOMOGENATES

Experiment No *	No tissue specimens	Concentration of homogenate (per cent)	Average % insulin $I^{131}$ degraded		
			Liver	Kidney	Muscle
1	4	1	13.1	9.5	6.6
	4	3	32.8	20.5	5.0
2	5	5	32.1	30.1	11.5
3	5	7.5	62.3	51.3	19.7
	5	15	74.4	64.3	25

\* The 3 experiments were conducted at intervals separated by weeks and different batches of insulin  $I^{131}$  were used.

ducted, with different batches of insulin  $I^{131}$  and with animals of various sizes at widely separated intervals all of the results are recorded with reference to the diaphragm, to which is assigned a value of 100. It is seen that essentially all of the tissues caused some degradation of the hormone. Among the more active tissues were liver, pancreas, kidney, and testis. On the other hand plasma had no activity, and washed red blood cells had essentially none. The action of brain, diaphragm, gastrocnemius and lymph node was relatively small in the case of muscle however, its body mass is so great that its total action is very significant.

#### INSULIN $I^{131}$ DEGRADATION BY SLICES OF DIFFERENT TISSUES

For each experiment, approximately 200 mg of each of these tissues was obtained from each of 12 male rats. For each 100 mg of tissue 1.25  $\mu$ g of insulin  $I^{131}$  was added, as well as 10  $\mu$ g of amorphous insulin. Upon completion of the incubation which was conducted as with the homogenates, the medium was poured into 1 ml of 2 per cent plasma. Ten ml of 20 per cent TCA was then added, the precipitate was washed twice with 2 ml of 5 per cent TCA. The tissue was washed 4 times with 2 ml of saline. The washings were combined. Counts of radioactivity in the supernates and washes were as described earlier.

#### EFFECT OF INFECTION AND TISSUE DAMAGE ON INSULIN DEGRADATION

In view of the fact that, first, the daily insulin requirements for diabetics are greatly increased with infection and trauma, and second, essentially all

TABLE 2  
INSULIN I<sup>131</sup> DEGRADATION BY VARIOUS RAT TISSUE HOMOGENATES\*

Experiment No †	1			2		3		4		5		6	
Rat No ‡	1	2	3	4	5	6	7	8	9	10	11	12	13
Liver						464	423	570	657	616	761	249	252
Pancreas	251	257	264	278	299	316	328	364	414	859		736	582
Kidney										516	540	217	231
Kidney cortex	217	235	265	252	261	410	335	362	410	458	619	178	227
Testis	291	264	282	291	295	458	412	316	408	325	419	114	149
Small intestines	178	299	258	225	216	131	131	191	169	248	322	135	165
Stomach	141	183	144	191	199	329	232	204	237	460	825	384	337
Spleen	189	169	190	199	205	361	342	248	314	348	543	172	139
Heart	139	174	141	109	135	343	309	273	237	159	194	70	96
Diaphragm	100	100	100	100	100	100	100	100	100	100	100	100	100
Gastrocnemius muscle						143		84	119	75	87	45	45
Brain	102	94	101	110	100	222	249	115	108	45		19	12
Adipose	54	52	54	27	33	113	96	89		22	30	22	21
Thymus	149	145	132	164	178								
Lymph nodes	102	81	71	80	92								
Red blood cells	5	2	1	4	7								
Plasma	0	0	0	0	0								

\* All values are related to the action of diaphragm which was arbitrarily given a value of 100

† The various experiments were conducted over an interval of 3 years (see text)

‡ In rats 1 to 9 100 to 150 mg of tissue was used per beaker in rats 10 to 13 15 mg in all instances the ratio of tissue weight to insulin weight was 10:1. In the experiments where only 15 mg of tissue homogenate was used 100 mg of bovine plasma albumin was added in order to reduce adsorption of the hormone to glass

tissues can degrade insulin we were prompted to ascertain whether increased insulin degradation was demonstrable under these circumstances

#### *Effect on Degradation of Insulin I<sup>131</sup> in Vitro of Serum from Diabetics with Infection*

As previously mentioned normal serum has been shown not to degrade insulin, but it was considered possible for the serum of infected diabetics to act differently. Therefore through the courtesy of Joseph Crampton serum was obtained from 2 diabetics each of whom was suffering from a carbuncle the development of which caused the need for about twice the usual daily dosage of insulin. Incubation of 1 ml of serum with insulin I<sup>131</sup> for 1 hour like that of serum of 4 normals studied simultaneously, caused no degradation of the hormone

#### *Degradation of Insulin I<sup>131</sup> in Diabetics and Nondiabetics with Infection*

Insulin I<sup>131</sup> was injected intravenously as described by Welsh *et al*<sup>7</sup> and plasma samples were obtained 1 hour later from 11 diabetics and 8 nondi-

betics with infection. Approximately one half of each group of subjects had fever at the time of the test, in a few patients the infection was severe (for example, pneumonia). The amount of TCA precipitable radioactivity in the serum 1 hour after insulin  $I^{131}$  administration was compared with large series of noninfected diabetics and nondiabetics.<sup>7</sup> There was no evidence that the infections were associated with increased degradation. In 2 diabetic patients the test was repeated when the subject was free of infection. In one, the results of the 2 tests were identical, in the other, there was actually less degradation with the infection because there was more binding to plasma globulin.

In view of the fact that there were many variables that might have influenced the insulin degradation, the problem was studied more specifically in animals and *in vitro*.

#### *Effect of Staphylococci on Insulin $I^{131}$ Degradation in vitro*

Because of the fact that staphylococci are such common offenders in diabetics, a total of 5 *in vitro* experiments was conducted with 2 highly virulent strains of this organism.

*Experiment 1* A heavy broth culture of staphylococci was centrifuged, washed twice with saline and then suspended in saline. One half of the material was subjected to supersonic vibration for 45 min to effect cellular disruption. The material was then recentrifuged, and part of the supernatant was tested as such whereas another part was boiled before testing. Thus there were 3 preparations of washed staphylococci (1) whole cells (2) supernatant from disrupted cells and (3) a portion of the second preparation that had been boiled. Each preparation was incubated for 5 min with insulin  $I^{131}$  and 4 mg of amorphous insulin alone or with the insulins plus liver insulinase. As the results shown in TABLE 3 indicate none of the preparations of staphylococci caused insulin degradation. Indeed the boiled staphylococcal supernatant actually decreased the degradation by the insulinase. This may have resulted from increased binding of insulin  $I^{131}$  to the boiled staphylococcal products.

TABLE 3  
EFFECT OF STAPHYLOCOCCI ON INSULIN  $I^{131}$  DEGRADATION

Insulin $I^{131}$	Liver enzyme	Other	Per cent degradation*
+			1.1
+	+		12.7
+		Washed staph	1.1
+	+	Washed staph	13.0
+		Sonified staph	1.4
+	+	Sonified staph	11.6
+		Boiled sonified staph	1.4
+	+	Boiled sonified staph	7.3

\* Average of triplicate determinations.

*Experiment 2* In this experiment the incubation was conducted at pH 6.0 and 7.5, only the latter was tested in the previous experiment. In this experiment there was used 80 mg (dry weight), per incubation flask, of staphylococcal products in the supernatant and 16 mg of the whole organisms. Again, the staphylococci caused no degradation.

*Experiment 3* The staphylococci in this and the next 2 experiments were obtained from one of the aforementioned diabetics with carbuncles and increased insulin requirements. The bacterial material was prepared as previously described. However the incubations were with only 0.025 as much amorphous insulin and were for 1 hour instead of for 5 min. The determinations were at pH 7.5 and 6.0. Again, no evidence of degradation by the staphylococci was observed.

*Experiment 4* The previous experiment was repeated, using a much greater concentration of staphylococci or their products and with one half as much amorphous insulin. No degradation from the bacteria was observed.

*Experiment 5* Experiment 4 was repeated except that 10 mg of bovine plasma albumin was added to each flask in order to minimize adsorption of insulin I<sup>131</sup> to glassware and/or products in the medium. There was no significant change in the results.

#### *Effect of Staphylococcal Infection in Rabbits on Insulin I<sup>131</sup> Degradation by Muscle*

All rabbits used were large white males.

*Experiment 1* Tourniquets consisting of a rubber band and a copper wire were placed high on the right thigh of each of 2 rabbits. Seven hours later the tourniquets were removed, and the gastrocnemius as well as surrounding structures were injected with 0.2 cc of a heavy suspension of staphylococci obtained from a diabetic patient, as described earlier. Five hours thereafter the animals were killed by a blow on the head. About 12 gm of traumatized and staphylococci injected muscle was removed, as well as a comparable amount of muscle from the other hind leg. After weighing, this material was homogenized in approximately 40 ml of 0.1 M Tris in a Waring Blendor. Following centrifugation the supernatant was removed, and 2 ml was incubated for 30 min with 1 ml of insulin I<sup>131</sup> containing 5  $\mu$ g of amorphous insulin. The amount of insulin I<sup>131</sup> degraded was determined in the manner described previously. There was no difference in the degradation caused by the muscle extract from the 2 legs.

*Experiment 2* An experiment similar to that above was conducted with 5 rabbits, but 1 ml of a heavier suspension of the staphylococci was injected into each hind leg. The supernatants from the muscle homogenate each contained 22 mg of solids per 1 ml. Each incubation flask contained 13  $\mu$ g of insulin. There was no difference in the degradation caused by the various extracts.

#### *Effect of Staphylococcal Infection in Rabbits on Insulin I<sup>131</sup> Degradation by Serum*

In the same white male rabbits described in the previous section, serum specimens were studied. About 15 ml of blood was withdrawn before the

tourniquets were applied and a few minutes after removing the tourniquets and massaging the leg in the second experiment an additional blood specimen was withdrawn 20 hours after initiation of the experiment. Two ml of serum was incubated for 15 min with 4 or 13  $\mu$ g of insulin in 1 ml Tris along with a small amount of insulin I<sup>131</sup>. None of the serum specimens caused degradation of the hormone.

#### *Effect of Staphylococcal Infection in Rats on Insulin I<sup>131</sup> Degradation in vitro*

Under ether anesthesia each of 6 male rats weighing approximately 250 gm was injected intraperitoneally with 0.5 ml of a heavy suspension of staphylococci obtained from the previously discussed diabetic patient. Six days later following a 24 hour fast each rat received 30  $\mu$ c of insulin I<sup>131</sup> in a tail vein. Thirty minutes thereafter under ether blood was removed from the aorta in a heparinized syringe. The amount of degraded and undegraded insulin was measured. An average of 49 per cent of the insulin I<sup>131</sup> was degraded and in another group of 6 rats treated in the same manner except for the injection of staphylococci there was a 50 per cent degradation of insulin I<sup>131</sup>.

#### *Effect in Rats of Injection with Fecal Contents on Insulin I<sup>131</sup> Degradation in vitro*

Five ether anesthetized rats were injected subcutaneously with 2 cc of a heavy fecal suspension in water. Eighteen hours later insulin I<sup>131</sup> was injected into the tail vein as in the previous experiment. The average degradation was 43 per cent whereas in 5 controls treated in the same manner except for the injection of fecal suspension the average degradation was 42 per cent.

#### *Effect of Necrosis of a Rabbit Leg on the Capacity of the Serum or Damaged Muscle to Degrade Insulin I<sup>131</sup> in vitro*

*Experiment 1* A rubber band and a copper wire were wrapped tightly around the upper part of the right hind leg of each of 2 rabbits. Five hours later the tourniquet was removed and the leg massaged for 2 min. Approximately 15 ml of blood was withdrawn from the heart before the tourniquets were applied as well as 2 min and 20 min after their removal. To 1 ml of serum was added 4 mg of amorphous insulin, 10  $\mu$ g insulin I<sup>131</sup> and 2 ml of Tris buffer. The mixture was incubated for 1 hour and the amount of insulin I<sup>131</sup> degraded was measured. None of the serum specimens promoted degradation.

*Experiment 2* Under ether anesthesia the right thighs of 7 rabbits were severely crushed with a hammer. This procedure subjected the animals to severe stress as evidenced by the fact that many died. The serum from blood drawn before the crushing 2.5 hours and 8 hours later when incubated for 15 min with insulin I<sup>131</sup> caused no degradation of the latter. About 10 gm of muscle was removed from each thigh and homogenized in saline. One aliquot of the supernatant from a 25 per cent muscle homogenate was adjusted to pH 7.5 with Tris buffer and another to pH 6.0 with phos-

phate One ml of each of these mixtures was incubated for 15 min with 30  $\mu$ g insulin and insulin I<sup>131</sup> in 1 ml of water All tests were conducted in duplicate The amount of insulin I<sup>131</sup> degraded by the traumatized and nontraumatized muscle extracts was comparable Experiments with the control muscle were conducted using twice or 0.5 the concentration of the muscle extract used above The degradation of insulin I<sup>131</sup> was proportionate to the amount of extract

*Effect of the Crushing of a Rat Leg upon the Degradation of Insulin I<sup>131</sup> in vivo*

Under ether anesthesia the right hind legs of 6 male rats were severely crushed with a hammer The process was repeated 4 days later and the animals were then fasted for 24 hours Insulin I<sup>131</sup> was injected into a tail vein and after 30 min blood was removed from the aorta and analyzed for degraded and undegraded hormone as previously described Six other rats were handled exactly in the same manner excluding the trauma The average amount of degraded insulin I<sup>131</sup> in the controls was 50 per cent and in the traumatized group 55 per cent ( $p < 0.001$ ) Three months later the experiment was repeated with 6 rats in similar manner except that only one traumatization was inflicted 24 hours before sacrifice and the rats were not fasted The amount of degradation was 47 per cent in the traumatized group and 41 per cent in the controls ( $p < 0.01$ )

EFFECT OF INSULIN I<sup>131</sup> DEGRADATION OF CONDITIONS PRESUMABLY INFLUENCING INSULIN SECRETION

Many chemical agents in the body in some manner exert a contra-insulin action Since insulinase markedly degrades insulin we were prompted to investigate in rats the effect on the insulinase action of many individual factors listed in TABLE 4 that influence carbohydrate metabolism and presumably the secretion of insulin In each individual experiment there were 6 rats except for one alloxanized group and the adrenalectomized group each of which included 4 rats Thus there were 116 experimental rats with completed studies and 60 controls The high fat diet consisted of a ratio of 1 gm of peanut oil to 1 gm of ground Purina Fox Chow \* The high carbohydrate diet consisted of 3 parts of fox chow to 7 parts sucrose and the high protein diet contained 7 parts casein per 3 parts of fox chow thus each of the 3 diets contained more than 75 per cent of its calories as fat carbohydrate or protein Twelve gm rat/day was served and all was devoured The phlorrhizinated and alloxanized rats showed persistent glycosuria Ultralente insulin was injected subcutaneously once daily For the experiments with inactive insulin an ultralente preparation was treated with cysteine Prednisolone acetate was injected subcutaneously once daily As shown in TABLE 4 some rats were of the Sprague Dawley strain and some were Wistars some were fasted and others not some were treated on the day of sacrifice and others not All rats were weighed at the beginning of the experiment (approximately 250 gm) and on the day of sacrifice All of the procedures were rigorous especially the prednisolone and insulin

\* Product of Purina Mills Buffalo N. Y.





administrations. A number of rats in these groups died. The alloxanized starved and prednisolone treated rats lost a great deal of weight. Those on high protein or high fat diets and those phlorhizinized lost a small amount. The adrenalectomized, hypophysectomized and high carbohydrate group did not gain. Each rat was decapitated and the liver rapidly removed, washed in 0.1 M Tris buffer, blotted on moist filter paper and weighed. The liver was homogenized in Tris. A 1 ml aliquot containing 15 mg liver (wet weight) was incubated at 37° C with 90 µg of insulin as well as insulin I<sup>125</sup> in 1 ml Tris for 5 min. Fifteen seconds before the end of incubation 1 ml of 2 per cent plasma was added. The reaction was stopped by the addition of 3 ml of 20 per cent TCA. The mixture was stirred, allowed to stand for at least 30 min, then centrifuged, decanted and the residues washed twice with 5 per cent TCA. The washes were combined and the radioactivity of the supernatants as well as the precipitates measured as described earlier. Since different intervals elapse during the preparation of the liver homogenates, studies were first conducted at 4° C to determine such effects on insulinase activity. Fifteen rats each weighing about 300 gm were decapitated and at 0, 2, 4, 6 and 8 hours thereafter the livers from 3 animals were removed and the insulinase determined; no significant difference was found.

Compared with the controls which were of comparable body weight at the beginning of the experiments, the total liver weights were significantly less in the following groups: adrenalectomy, hypophysectomy, alloxan, high fat and starved; they were increased in the prednisolone and high protein groups. The percentage of water was decreased in the adrenalectomy, hypophysectomy and the prednisolone groups.

The averages of the other major observations are shown in TABLE 4. Considering the marked changes in metabolism, there were surprisingly few alterations in liver insulinase activity. There was an increase in the degradation of the liver homogenate of one high fat and one alloxan group when the calculations were based on a liver dry weight ratio, but not by the other standards. On a liver wet weight basis there was one insulin group that had an increased degradation and one prednisolone group that had a

weight. Considering the radical metabolic changes that were found to be induced by the various procedures, the relatively few abnormalities in liver insulinase were surprising.

#### GLUCAGON I<sup>125</sup> DEGRADATION

Using glucagon labeled with I<sup>125</sup> in the manner described previously<sup>8,9</sup> it was found that homogenates of pancreas, liver, kidney, cortex and muscle broke down this labeled hormone. Indeed, in concentrations of homogenates from 0.05 to 0.4 per cent and 5 µg of glucagon I<sup>125</sup> the relations were essentially linear (FIGURE 1). Studies with muscle and liver, however, showed

that the effect tended to plateau when more than 1 per cent concentration was used

Glucagon I<sup>125</sup> degradation by acetone precipitates from liver homogenate is inhibited by many of the same compounds that inhibit the insulin I<sup>125</sup> degradation<sup>10-11</sup> However there was a different degree of inhibition in some instances For example the following inhibited insulin I<sup>125</sup> degradation more than glucagon I<sup>125</sup> benzoyl L tyrosine ethyl ester acetyl L tryptophan ethyl ester tannic acid dithiouracil bis (dimethylcarbamyl)

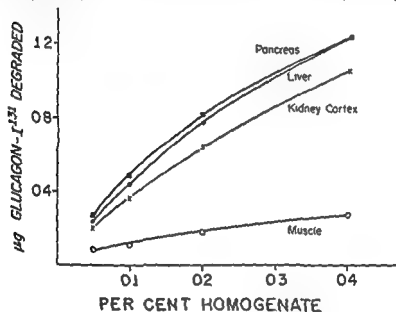


FIGURE 1 The degradation of glucagon I<sup>125</sup> by varying concentrations of tissue homogenates

disulfide and 1 butyl 3 o aminophenylsulfonylurea A comparable degree of inhibition of degradation of the 2 hormones was observed with phenethyl biguanide and 1 phenyl 3 phenylsulfonylurea

#### DISCUSSION

Before discussing our results consideration will be given to some of the biochemical factors involved in insulin and glucagon degradation It has long been known that glutathione (GSH) and other such reducing substances inactivate insulin<sup>12</sup> indeed the hormone is inactive when only one third of the disulfide linkages have been reduced<sup>13-14</sup> Insulin is inactivated

could be glutathione since it definitely degrades insulin I<sup>125</sup>, however,

reduction is not a prerequisite for insulin  $I^{121}$  degradation because an acetone precipitate of liver homogenate degrades insulin  $I^{121}$  even after dialysis for 48 hours<sup>17</sup> Such a preparation causes much less degradation than an undialyzed one or a dialyzed one to which glutathione has been added,<sup>18</sup> under certain conditions glutathione activity synergizes enzymatic degradation and conversely, enzymatic action stimulates degradation by glutathione<sup>18</sup> Rat liver has been shown to contain an enzyme designated as glutathione reductase<sup>19</sup> that catalyzes the reduction of oxidized glutathione (GSSG) This TPN-linked GSSG reductase system does not act directly on insulin but promotes regeneration of GSH that has been oxidized by insulin it increases the effectiveness of a given amount of glutathione There is evidence suggesting that there is a second enzyme in the liver that directly reduces insulin probably by promoting transfer of hydrogen from GSH to

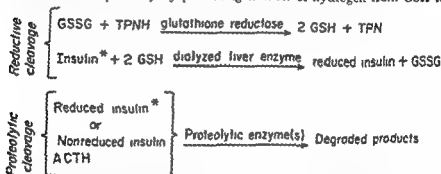


FIGURE 2 Liver enzymes that may be involved in the degradation of insulin and other hormones Somatotropin prolactin vasopressin or oxytocin also may engage in the reactions indicated by the asterisks

insulin<sup>18</sup> These observations are in keeping with a preliminary report by Racker<sup>10</sup> suggesting that enzymatic reduction of insulin by liver might occur In conclusion as diagrammed in FIGURE 2 it appears that reduction of an enzymatic and nonenzymatic type causes the inactivation and degradation of insulin However this presumably is not the only method of degradation, because many observations have suggested<sup>1, 2</sup> that the liver contains one or more enzyme systems that cause proteolytic degradation of insulin The use of insulin  $I^{121}$  has been of great assistance in these studies<sup>3, 4, 21, 22</sup> Mirsky has presented the hypothesis that there is a single enzyme in liver that has a high degree of specificity for the degradation of insulin<sup>23</sup> This may prove to be the case, but it has not been possible to establish it as a fact because of the crudity of the enzyme preparations that have always been used<sup>24</sup> such preparations probably contain several enzymes and act on a number of substrates other than insulin Tomizawa and Halsey<sup>25</sup> have recently isolated a protein fraction from beef liver apparently pure by starch block electrophoretic and ultracentrifugal patterns, which degrades insulin  $I^{121}$  However glucagon  $I^{121}$  degradation is not demonstrated by the usual method The specific mechanism of degradation of insulin is under investigation

In the studies conducted with crude liver extract it has been shown that the degradation of insulin I<sup>125</sup> is decreased by casein  $\alpha$  corticotropin glucagon somatotropin and certain other proteins<sup>23</sup>. On the other hand other proteins for instance albumin<sup>22, 24</sup> exert no effect. Actually many protein hydrolyzates and amino acids as well as a vast number of other compounds inhibit insulin I<sup>125</sup> degradation<sup>2, 10, 1, 26, 27</sup> some of the inhibitors are competitive and some are noncompetitive. The same crude liver enzyme preparation that degrades insulin I<sup>125</sup> has been shown to degrade glucagon I<sup>125</sup> also<sup>4, 28, 29</sup>. The degradation of each hormone is also inhibited by  $\alpha$ -corticotropin  $\alpha$ -casein and somatotropin nonlabeled glucagon or insulin<sup>4, 30</sup> phenethylbiguanide 1 butyl 3 *o* aminophenylsulfonylurea 1 phenyl 3 phenylsulfonylurea bis (dimethylcarbamyl) disulfide dithiouracil tannic acid acetyl L tryptophan ethyl ester benzoyl L tyrosine ethyl ester *p*-chloromercuribenzoic acid and many other compounds including hydrolyzates of the aforementioned proteins<sup>30</sup>. The fact that the enzyme action is blocked by *p*-chloromercuribenzoic acid and other agents that react with sulfhydryl action T insulin I<sup>125</sup>

enate subjected to differential centrifugation<sup>4, 28</sup>. Each labeled hormone is broken down by most tissues of the body<sup>1, 4</sup> the liver and kidney being particularly active.

In spite of the many similarities of enzymatic degradative actions on insulin and glucagon a number of differences have been demonstrated. For example GSH greatly increases the amount of degradation of insulin I<sup>125</sup> caused by a dialyzed liver enzyme preparation whereas it has little effect on glucagon I<sup>125</sup> degradation. Iodoacetate and cupric ion agents that react with glutathione inhibit insulin degradation more than glucagon<sup>30, 31</sup>. Aqueous or bicarbonate extracts of rat liver lose their insulin hydrolyzing action on standing overnight in the cold on dilution or on blending for 2 min. these procedures do not have much effect on the amount of glucagon degradation<sup>4</sup>. Citrate increases insulin degradation but does not affect that of glucagon<sup>22, 32</sup>. One liver preparation has been reported<sup>33</sup> to inactivate glucagon but not insulin. Conversely as mentioned earlier the purified enzyme isolated by Tomizawa and Halsey degrades insulin I<sup>125</sup> but not glucagon I<sup>125</sup>.

In analyzing the studies reported thus far it appears (FIGURE 2) that in insulin degradation enzymatic and nonenzymatic reductions are important whereas the reductive processes are not involved in glucagon degradation since glucagon does not contain disulfide bonds. However proteolysis seems to occur in the degradation of each hormone<sup>29, 3, 34</sup>. We do not know how and to what extent the reductive and the proteolytic reactions are related. It is possible that other disulfide-containing hormones such as somatotropin prolactin oxytocin and vasopressin may like insulin be degraded by both reductive and proteolytic reactions whereas the degradation of corticotropin like that of glucagon may involve proteolysis only.

As previously noted most tissues have the capacity to degrade glucagon I<sup>125</sup>

as well as insulin  $I^{121}$  in each instance the liver kidney pancreas and testis are among the most active. Not much is known about the degradative systems of tissues other than the liver. Muscle contains a nondialyzable heat labile system.<sup>39</sup> Corticotropin somatotropin oxidized insulin  $\alpha$  casein and glucagon inhibit the degradation of insulin  $I^{121}$  by muscle and enhance the glucose uptake by rat diaphragm.<sup>40</sup> The enhancing effect of glucagon on insulin increased glucose uptake had been noted earlier by Randle.<sup>41</sup> Crude extract of beef anterior pituitary also contains an enzyme system that causes insulin  $I^{121}$  degradation.<sup>42</sup> It has been shown that insulin  $I^{121}$  degradation *in vitro* is reduced in hypophysectomized rats.<sup>43</sup>

In studies with insulin  $I^{121}$  it was shown that tissue slices were much less active than homogenates although the relative order of effectiveness of the different tissues was similar. This apparently is due to the liberation of the large quantities of enzyme(s) from the soluble intracellular fraction.

Once insulin is bound to protein it is less subject to degradation.<sup>44</sup> Indeed the binding of insulin  $I^{121}$  to plasma globulin accounts for the abnormally slow degradation of insulin  $I^{121}$  in subjects who have had insulin treatment for several weeks or longer.<sup>45</sup> Diabetics not treated with insulin apparently have a normal rate of insulin degradation. Even those with infection were found to have a normal rate of degradation of insulin  $I^{121}$  *in vivo* and sera from such patients incubated with insulin  $I^{121}$  did not degrade insulin  $I^{121}$ . Many studies were conducted to investigate further whether the increase in dosage of insulin required by diabetics with infection or trauma was due to increased insulin degradation. However there was no degradation produced by sera of patients or animals infected with staphylococci and no increased degradation *in vitro* with staphylococcal infection or in rats infection caused by the subcutaneous injection of fecal suspensions. Whole or fragmented staphylococci incubated with insulin  $I^{121}$  did not degrade it. Severe traumatization of the hind leg of rabbits did not cause the presence of insulin  $I^{121}$  degrading material in the serum whether or not there also was infection of the muscle. With muscle traumatization in rats there was demonstrated an increase in insulin  $I^{121}$  degradation *in vitro*.

Because of the fact that insulin  $I^{121}$  is degraded very rapidly in the body and because the liver has a large capacity for this action and has the first chances to react with endogenous insulin the quantity of insulinase activity in liver was studied in rats subjected to one of several vigorous procedures thought to influence significantly the amount of insulin secreted. These included starvation phlorrhizination alloxan diabetes injections of prednisolone or insulin hypophysectomy adrenalectomy and diets high in carbohydrate fat or protein. Starvation was previously reported to decrease liver insulinase activity.<sup>46</sup> We found that it decreased the total liver insulin  $I^{121}$ -degrading capacity. A few abnormalities were produced by other factors but we were impressed by the fact that they were relatively small considering the strenuous nature of the procedures. These observations raise the question as to how extensively the insulinase system is involved in the homeostasis for carbohydrate metabolism. However it always has a potent insulin degrading capacity and there is evidence that

the greater the insulin content in the body <sup>1, 2</sup> the larger is the total amount degraded

### SUMMARY AND CONCLUSIONS

Essentially all tissues possess the capacity to degrade insulin I<sup>131</sup> and glucagon I<sup>131</sup> rapidly liver pancreas kidney and testis are among the most active Much more information must be obtained before the mechanism(s) of degradation are established but in the case of liver present information suggests that insulin is degraded by enzymatic and nonenzymatic reduction as well as by proteolysis whereas glucagon degradation is affected by the latter process alone Tissue homogenates degrade insulin much faster than tissue slices Many different compounds inhibit the degradation of insulin and glucagon some by competitive and others by noncompetitive reactions Staphylococcal infections in patients rats and rabbits do not cause the serum to degrade insulin I<sup>131</sup> Indeed neither whole staphylococcal cells nor disrupted cells cause degradation of the hormone Traumatization of the rabbit leg does not cause the serum to degrade insulin nor does it increase the degradative capacity of muscle However traumatization of rat leg causes an increased degradation of insulin I<sup>131</sup> *in vivo* Of many procedures assumed to influence significantly the amount of insulin secreted few change the degradative capacity of liver to a biologically significant degree The conditions tested were starvation insulin or prednisolone injections hypophysectomy adrenalectomy alloxan diabetes phlorhization and diets high in carbohydrate fat or protein Additional study is needed to determine the role of insulin degrading enzymes in homeostasis and in the pathogenesis of diabetes

### ACKNOWLEDGMENTS

We thank Henry H Tomizawa and H T Narahara for helpful advice

grateful to Elaine Henley for assistance in some of the studies with patients

### REFERENCES

- 1 WILLIAMS R H 1956 Insulin distribution and degradation Metabolism 5 128
- 2 MIRSKY I A 1956 The role of insulinase and insulinase inhibitors Metabolism 5 138
- 3 MIRSKY I A 1956 Insulinase insulinase inhibitors and diabetes mellitus Recent Progr in Hormone Research 11 429
- 4 SALTER J M I W I DAVIDOV & C H BERT 1957 The pathologic effects of  
Distribution and degradation  
R H WILLIAMS 1955 The  
J Biol Chem 214 295  
W Cox 1956 Insulin I<sup>131</sup>
- 5
- 6
- 7
- 8 NARAHARA H T & R H WILLIAMS 1957 Degradation of glucagon I<sup>131</sup> by rat  
tissues and its localization 60 295

- 9 COY R W E D HENLEY H T NARAHARA & P P VAN ARSDEL 1957 Studies on the metabolism of glucagon  $I^{131}$  in rats *Endocrinology* 60 277
- 10 WILLIAMS R H & F B MARTIN 1956 Compounds inhibiting insulin degradation *Diabetes* 5 451
- 11 WILLIAMS R H F B MARTIN L D HENLEY & H C SWANSON 1959 Inhibitors of insulin degradation *Metabolism* In press
- 12 DU VIGNEAUD V A FITCH L PEKAREK & W W LOCKWOOD 1931 1932 The inactivation of crystalline insulin by cysteine and glutathione *J Biol Chem* 94 233
- 13 WINTERSTEINER O 1933 The action of sulphhydryl compounds on insulin *J Biol Chem* 102 473
- 14 FRANKEL CONRAT J & H I RAENKEL CONRAT 1950 The essential groups of insulin *Biochim et Biophys Acta* 5 89
- 15 LEHMANN H & H SCHLOSSMANN 1939 The action of cell free muscle extract on insulin *J Physiol* 94 15P
- 16 MIRSKY I A & R H BROWN KAHN 1949 The inactivation of insulin by tissue extracts I The distribution and properties of insulin inactivating extracts (insulinase) *Arch Biochem* 20 1
- 17 NARAHARA H T H H TOMIZAWA & R H WILLIAMS 1956 Sulphydryl factors in degradation of insulin  $I^{131}$  by liver extracts *Proc Soc Exptl Biol Med* 91 718
- 18 NARAHARA H T & R H WILLIAMS Reduction of insulin by an extract of rat liver In preparation
- 19 RALL T W & A I LEHNINGER 1952 Glutathione reductase of animal tissues *J Biol Chem* 194 119
- 20 RACER R 1953 Metabolism of thioesters of glutathione *Federation Proc* 12 711
- 21 FERREBE J W B M JOHNSON J C WITHOEFER & J W GARDELLA 1951 Insulin and adrenocorticotropin labeled with radio-iodine *Endocrinology* 48 277
- 22 MIRSKY I A G PERISUTTI & F J DIXON 1955 The destruction of  $I^{131}$  labeled insulin by rat liver extracts *J Biol Chem* 214 397
- 23 MIRSKY I A & G PERISUTTI 1957 The relative specificity of the insulinase activity of rat liver extracts *J Biol Chem* 228 77
- 24 TOMIZAWA H H & R H WILLIAMS 1955 Studies on the specificity of an insulin inactivating system of the liver *J Biol Chem* 217 683
- 25 TOMIZAWA H H & Y HALSEY 1958 Isolation of an insulin degrading enzyme from beef liver, Submitted for publication
- 26 MIRSKY I A & G PERISUTTI 1957 Insulinase inhibitory activity of protein hydrolysates *Proc Soc Exptl Biol Med* 94 589
- 27 WILLIAMS R H & M K BERG 1956 Inhibition of insulin degradation by amino acids and related compounds *Proc Soc Exptl Biol Med* 92 20
- 28 TYBERGHEIN J M H H TOMIZAWA & H H WILLIAMS 1956 Glycogenolytic action of glucagon as influenced by insulin and other compounds *J Biol Chem* 212 945
- 29 NARAHARA H T H H TOMIZAWA R MILLER & R H WILLIAMS 1955 Intracellular distribution of an insulin activating system of liver *J Biol Chem* 217 675
- 30 KENY A J 1956 Inactivation of glucagon by tissues *in vitro* *Am J Physiol* 186 419
- 31 KENY A J 1958 The proteolysis of glucagon and other peptides by the rat liver *in vitro* *Biochem J* 80 32P
- 32 DAVIS N C R JINNS & I A MIRSKY 1958 Some ion effects on activity of rat liver insulinase *Federation Proc* 17 822
- 33 SNEDECOR J G & W B MACGRATH JR 1952 The effect of insulinase upon the hyperglycemic factor of the pancreas *Anat Record* 113 588
- 34 BERSON S A R S YALOW & B W VOLK 1957 *In vivo* and *in vitro* metabolism of insulin  $I^{131}$  and glucagon  $I^{131}$  in normal and cortisone treated rabbits *J Lab Clin Med* 49 331

- 35 NARAHARA H T & R H WILLIAMS 1958 Effect of proteins added *in vitro* upon insulin degradation and glucose uptake by muscle J Biol Chem In press
- 36 RANDLE P J 1956 Discussion of paper by J L R CANDELA & J J CANDELA Inhibitory effect of glucagon on the insulin glucose uptake of the isolated diaphragm of the rat Ciba Foundation Colloquia on Endocrinology 9 199 Little Brown Boston Mass
- 37 NARAHARA H T & R H WILLIAMS 1958 Degradation of insulin I<sup>125</sup> by an extract of anterior pituitary gland Am J Physiol 193 476
- 38 TIGEE N J & R H WILLIAMS 1955 The fate of insulin in altered metabolic states Diabetes 4 8
- 39 YALOW R S & S A BERSON 1957 Apparent inhibition of liver insulinase activity by serum and serum fractions containing insulin binding antibody J Clin Invest 36 648
- 40 BERSON S A R S YALOW A BAUMAN M A ROTHCHILD & N K NEWERLY 1956 Insulin I<sup>125</sup> metabolism in human subjects demonstration of insulin binding globulin in the circulation of insulin treated subjects J Clin Invest 35 170
- 41 BROTH KAHN R H & I A MIRSKY 1949 The inactivation of insulin by tissue extracts II The effect of fasting on the insulinase content of rat liver Arch Biochem 20 10



# DETERMINATION OF THE RATE OF INSULIN DESTRUCTION IN VIVO\*

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Insulin given by intravenous injection disappears rapidly from the plasma. The half time of insulin labeled with iodine 131 ( $I^{131}$ ) in the intravascular space has been shown to be approximately twenty five minutes<sup>1</sup>. The rapid decline in plasma concentration is the result of several events that occur simultaneously: first, insulin undergoes proteolytic destruction in the liver and kidney;<sup>2</sup> second, insulin may be bound or sequestered by tissue cells;<sup>3</sup> and third, insulin may be dispersed in the compartments of body water. The experiments reported in this paper were designed to determine the extent to which each of these processes affects insulin disappearance.

## Methods

Normal male rabbits were used in this study. Animals were sacrificed at the end of each experiment by intravenous injection of lethal quantities of pentobarbital. Complete urine collections were obtained by excision of the bladder. The experiment was discontinued if spontaneous voiding occurred. Eviscerated animals were prepared by removal of the intestinal tract from the lower esophagus to the rectum including the spleen and pancreas, under barbital narcosis and ether anesthesia. The arterial blood supply to the liver was ligated and sectioned. Barbital narcosis was continued in these animals throughout the experiment. Nephrectomy was performed either alone or in association with evisceration as described in the experimental detail.

Isotonic sterile saline was added to radioinsulin† to form a concentration of approximately 5  $\mu$ c/ml. The solution was stored at 10° C.

At the start of each experiment each animal was given intravenous injection of 2 ml of a 10 per cent solution of NaI. A 20 min period of circulation of the NaI was allowed in order to block uptake of free  $I^{131}$  by the thyroid gland. Crystalline zinc insulin was then given by intravenous injection to some of the animals. Radioactive insulin was administered intravenously 10 min after the injection of crystalline zinc insulin. A 50 per cent solution of glucose (1 to 2 ml  $I/V$ ) was used to prevent hypoglycemia.

The volume of injected radioinsulin was measured accurately in a syringe calibrated by means of a threaded brass rod: the plunger of the syringe was advanced by a screw. In order to determine the quantities of insulin injected, an aliquot of the material that had been drawn into the syringe was

\* The work reported in this paper was supported in part by a grant from the American Cancer Society, New York, N. Y. and by Research Grant A 2041 and Graduate Training Grant 24 5136 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

† Insulin  $I^{131}$  was obtained from Abbott Laboratories, Oak Ridge, Tenn.

added to 50 ml of pooled human plasma (serum phantom). Analysis of the radioactivity in this serum phantom gave an accurate measure of the characteristics and quantity of radioactive material injected into the rabbit.

The analysis of both the serum phantom and of the rabbit serum were carried out by the same technique on 0.1 ml of serum. Intact insulin was isolated by electrophoresis or hydrodynamic flow.<sup>14</sup> The precipitable radioactivity was obtained by adding 1 ml of 10 per cent trichloroacetic acid (TCA) to 0.1 ml of serum. The precipitate was separated by centrifugation and the radioactive supernatant fluid was poured off. Determination of the quantity of total radioactivity was obtained by adding that contained in the TCA precipitate to that in the TCA soluble fractions. The quantity of total circulating radioactivity was also checked by placing 0.1 ml of serum on Whatman No. 3 filter paper as in the procedure for electrophoresis. However, in this instance the paper was placed immediately in 3 cc. KOH. Radioactivity in all samples was counted in a shielded well type scintillation crystal connected through a linear preamplifier to a scaler. The gamma emissions of the  $^{131}\text{I}$  were measured to eliminate errors of interference. All counts were carried out to sufficient numbers to reduce the counting error to less than 2 per cent.

TABLE 1  
PERCENTAGES OF DEVIATION FROM THE MEAN OF THREE CONSECUTIVE SERUM ANALYSES

Compartments	Deviation from mean			Average
Total radioactivity	+1	-3	+1	$\pm 1.4$
TCA precipitate	+1	0	-2	$\pm 1.0$
TCA soluble	+1	-3	+1	$\pm 1.4$
Insulin	-3	+2	+1	$\pm 2.0$
Average error per cent total count				$\pm 1.6$

Determinations of the radioactivity in the TCA precipitable and the TCA soluble insulin and of the total compartments were carried out in duplicate on both the serum of the rabbit and the serum phantom. Three consecutive serum phantoms were compared for accuracy. The deviations of determinations of each of the serum compartments from their mean are shown in TABLE 1. The average error introduced by each analysis was 1.6 per cent of the total counts.

### Results

It has been demonstrated that insulin labeled with  $^{131}\text{I}$  can be separated from radioactive proteins in serum more accurately by electrophoresis than by precipitation with trichloroacetic acid. The assumption that all radioactivity precipitated from the serum by trichloroacetic acid is biologically active insulin was not supported by previous work from this laboratory.<sup>1</sup> The importance of using a technique with which the compartments can be

clearly distinguished is shown in FIGURE 1. The first column shows the characteristics of the radioactive material assayed in serum immediately after its arrival from the manufacturer. Ninety two per cent of the radioactivity in solution can be precipitated by trichloroacetic acid. The remaining 8 per cent is in the TCA soluble fraction. Only 86 per cent of the total radioactivity can be isolated as insulin by electrophoresis. Subtraction of the quantity of radioactivity isolated as insulin by electrophoresis from the TCA precipitable fraction leaves the radioactive fraction due to biologically inactive material. Columns 2 and 3 show the characteristics of the material at 4 and 8 days, respectively. The insulin fraction is now 77 and 74 per cent of total radioactivity, the inactive fraction has risen from 4 to 15 and 17

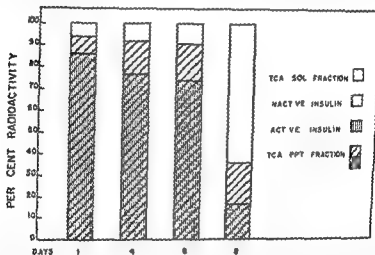


FIGURE 1. Characteristics of the radioactive solution. Radioactive solution stored at 10° C was added to serum on the days shown. Analysis was carried out immediately following mixing. Composition of the radioactivity in solution changes gradually with time. The fourth column shows the change in the characteristics of the solution kept at room temperature over an 8-day period.

per cent respectively over this period. The fourth column shows the characteristics of a part of the material that was separated from the original but left at room temperature. The insulin content is reduced to 17 per cent of the total radioactivity after 8 days; the TCA soluble fraction is 64 per cent of the total radioactivity. It is important to note that the characteristics of the radioactive solution may change completely. Unless the characteristics of the radioactive solution are followed, reduction in the insulin content as shown here will be undetected. This will alter the results obtained and studies done at different times will not be comparable.

The biologically inactive material migrates with the serum protein on electrophoresis of the serum. There is no detectable degradation of this fraction over a period of several hours and it continues to recirculate in the plasma.<sup>1</sup> Therefore the TCA soluble radioactivity that appears in the

plasma or serum following injection of the radioactive solution comes from the degradation of the insulin. These products of degradation will hereafter be designated as soluble  $I^{131}$ .

The rate of insulin destruction *in vivo* may be determined by the following technique. The amount of radioactivity injected as insulin  $I^{131}$  and the soluble  $I^{131}$  are measured by analyses of the serum phantom. At the end of the experimental period blood is drawn for determination of the serum concentration of insulin and soluble  $I^{131}$ . The bladder is removed from the intact animals for determination of soluble  $I^{131}$  that has been excreted in the urine. The soluble  $I^{131}$  resulting from insulin degradation that is not

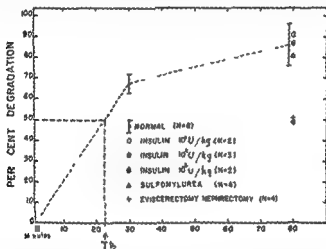


FIGURE 2. Insulin degradation in the rabbit. Degradation of radioinsulin injected in the rabbit has been studied under various conditions. Prior administration of insulin in dosages of  $10^1$  and  $10^2$  U/kg did not alter the degree of degradation. Sulfonylurea (tolbutamide, chlorpropamide) had no effect on degradation. Insulin in dosage of  $10^3$  U/kg in the intact animal decreased degradation to the level of that in the eviscerated nephrectomized animal.

excreted is calculated by multiplying the  $I^{131}$  content of the serum by the iodine space. The iodine space was found to be 26 per cent of body weight and is in agreement with previous determinations.<sup>6</sup> The intact insulin remaining at the end of the experiment is the difference between the injected radioactivity and the total degradation products.

The per cent of insulin degradation at 30 min and 80 min in the normal rabbit is shown in FIGURE 2. At 30 min after injection an average of 68 per cent of injected radioinsulin has undergone separation of the radioactive label. This is assumed to be the result of proteolytic destruction of the insulin molecule. The half time of insulin destruction = 22 min. This correlates closely with the half time of the disappearance of radioactivity from the plasma as previously reported.<sup>1</sup> At 80 min an average of 86

per cent of the injected radioinsulin has been degraded. This may be contrasted to the rate of degradation of insulin in the eviscerated nephrectomized rabbit. As shown in FIGURE 2 the degradation of insulin was decreased to 51 per cent at 80 min under these circumstances.

In order to slow the process of degradation in the normal rabbit, an attempt was made to saturate the degrading mechanisms with crystalline zinc insulin. Dosages of crystalline zinc insulin of 1, 10, 100 and 1000 U/kg body weight were injected 10 min prior to the injection of radioinsulin. The rate of insulin degradation was not influenced by quantities of insulin up to 100 U/kg body weight. When 1000 U of insulin/kg body weight were injected prior to the radioinsulin the rate of degradation was reduced to 48 per cent at

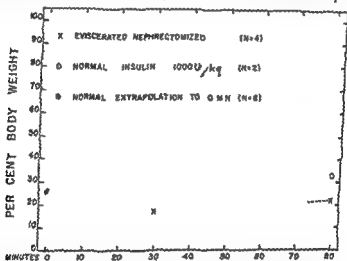


FIGURE 3 The apparent volume of distribution of insulin in the rabbit is equivalent to the volume of the extracellular space in the insulin treated (10<sup>3</sup> U/kg) and eviscerated nephrectomized rabbit. Previous determinations in the normal animal<sup>1</sup> are also shown for comparison.

80 min. It is clear that the degrading mechanisms for insulin are difficult to saturate and far exceed their physiological need.

The possibility that the sulfonylurea compounds act by decreasing insulin destruction is suggested by some of the studies done *in vitro* with enzyme systems.<sup>6</sup> On the other hand no change in the rate of disappearance of radioinsulin following tolbutamide administration was noted.<sup>7</sup> The effect of tolbutamide and chlorpropamide on insulin degradation has been studied with the present technique. As shown in FIGURE 2 there was no decrease in the rate of insulin destruction following the use of these drugs in the normal rabbit.

In order to clarify the role of tissue binding in the disappearance of insulin the space of distribution of insulin must also be determined. It is probable that tissue binding and the space of distribution are closely related. A determination of insulin space was made in earlier studies from this

laboratory in which it was assumed that the rate of degradation was constant from the time of injection. Following injection of radioinsulin determinations of the insulin content of serum were made between 30 and 80 min. after injection. The rate of insulin disappearance was extrapolated to zero time. The insulin content of serum at zero time was divided into the quantity of insulin given by injection. By this calculation the insulin space was found to be approximately 26 per cent of total body weight.<sup>1</sup> The insulin space was not altered by prior administration of sulfonylurea.<sup>2</sup>

The present technique has been applied also to the study of the insulin space. The quantity of intact insulin remaining at 30 or 80 min. divided by the insulin concentration per ml. of serum at the same intervals gives the apparent volume of distribution of the intact insulin. Insulin destruction by the liver and kidney was eliminated in these experiments in order to clarify the effect of the peripheral tissue on insulin disappearance. As shown in FIGURE 3 the insulin space was found to be 16 per cent of body weight at 30 min. and 22 per cent of body weight at 80 min. in the eviscerated nephrectomized rabbit. Also shown are the results obtained when degradation is decreased by the prior intravenous administration of insulin in a dosage of 1000 I. kg. The results in FIGURE 3 are in accordance with the previous estimations of the insulin space<sup>1, 3</sup> and are approximately the size of the extracellular space.

The implications of this apparent volume of distribution of insulin should be considered further. The quantity of insulin necessary to saturate the binding sites for insulin in muscle has been estimated by Stadie<sup>4</sup> to be approximately 1.5  $\mu$ g. of insulin/gm. of tissue. Under the conditions of the experiments reported here the availability of tissue binding sites must greatly exceed the available insulin. If intact insulin were captured by peripheral cells without degradation the quantity of insulin remaining in the periphery would increase while the concentration of circulating insulin would become progressively smaller. Therefore the apparent volume of distribution of the intact insulin would become progressively larger and finally become indeterminate. The apparent volume of distribution found in these experiments is inconsistent with this assumption. The radioactive insulin injected can be accounted for by the summation of the insulin subjected to proteolytic destruction and the insulin contained in extracellular water. This leads to the interpretation that the degradation of intact insulin is not prevented by tissue binding or sequestration. The similarity between the space occupied by intact insulin and the extracellular compartment suggests rather that insulin is degraded quickly after leaving the extracellular space. The clinical implication of these results is that intact insulin cannot be saved for future use by peripheral tissues.

#### Summary

Factors influencing the rate of disappearance of insulin from plasma have been studied by the use of insulin labeled with iodine 131 ( $I^{131}$ ). A technique has been employed that measures the amount of active intact insulin administered. Some change in the characteristics of the radioactive solution on

standing for short periods makes this necessary. In the intact animal an average of 86 per cent of the injected insulin was degraded in 80 min. The rate of degradation was not influenced by prior administration of sulfonyl urea compounds.

The apparent volume of distribution of insulin *in vivo* is equivalent to the volume of extracellular water. Sequestration of intact insulin without degradation is not a factor in the rapid disappearance of insulin from plasma. The radioactive insulin injected can be accounted for by the summation of the insulin subjected to proteolytic destruction and the insulin contained in extracellular water. Under the conditions of these experiments, there is no evidence that the degradation of intact insulin is prevented by tissue binding or sequestration.

### Acknowledgments

We thank Lorraine Conway and Beverly Smith for their technical assistance.

### References

1. SCOTT, G. W., T. E. PROUT, J. A. WEAVER & S. P. ASPER. 1959. A comparison of the behavior of insulin and insulin labeled with  $I^{131}$  in serum. *Diabetes* 7: 38.
2. ELOE, N. J., H. WILLIAMS & N. D. LEE. 1954. Distribution and degradation studies with insulin  $I^{131}$ . *J. Clin. Invest.* 33: 1252.
3. STADIE, W. C., N. HALOVAR & M. VALCHAY. 1952. Studies of insulin binding with isotopically labeled insulin. *J. Biol. Chem.* 199: 729.
4. BEYSON, S. A., R. S. YALOW, A. BAUMAN, M. A. ROTHCHILD & L. NEWERLY. 1956. Insulin  $I^{131}$  metabolism in human subjects: demonstration of insulin binding globulin in the circulation of insulin treated subjects. *J. Clin. Invest.* 35: 170.
5. BEYSON, S. A., R. S. YALOW, J. SORRENTINO & M. ROSWIT. 1952. The determination of thyroidal and renal plasma  $I^{131}$  clearance rates as a routine diagnostic test of thyroid dysfunction. *J. Clin. Invest.* 31: 141.
6. MIRSAY, J. A., G. PERISCTTI & D. DIENGOTT. 1956. The inhibition of insulinase by hypoglycemic sulfonamides. *Metabolism* 5: 156.
7. WEAVER, J. A., T. E. PROUT, G. W. SCOTT & S. P. ASPER. 1958. Role of insulin in acute hypoglycaemic action of tolbutamide. *Brit. Med. J.* 1: 425.
8. DRURY, D. R., W. A. KARASEK, B. BRITTON & A. A. HICK. 1958. Metabolism of insulin  $I^{131}$  in the extrahepatic tissues. *Am. J. Physiol.* 192: 501.
9. STADIE, W. C. 1957. *In Hormonal Regulation of Energy Metabolism*. 13. L. W. Kinsell, Ed. Thomas Springfield Ill.

## A COMPARISON OF THE INFLUENCE OF TOLBUTAMIDE AND SMALL DOSES OF INSULIN ON THE Splanchnic OUTPUT AND PERIPHERAL UPTAKE OF GLUCOSE IN MAN\*

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Considerable evidence has been offered to support the hypothesis that the administration of a sulfonylurea to a human subject or animal with an intact pancreas is followed by the release of a substance with hypoglycemic activity, presumably insulin from the pancreatic beta cells.<sup>1</sup> The refractoriness of fully alloxanized animals and the influence of the beta cell reserve on the character of the early hypoglycemic response to the drugs<sup>2</sup> are compatible with this hypothesis. In addition pancreatic vein blood from dogs given a sulfonylurea reduced the blood glucose concentration of both normal<sup>3</sup> and alloxan-diabetic<sup>4</sup> recipient dogs in cross circulation experiments and produced hypoglycemia in mice.<sup>5</sup>

The unqualified acceptance of insulin as the mediator of sulfonylurea induced hypoglycemia has been tempered by the failure of many investigators to demonstrate an insulinlike effect of the drugs on the peripheral tissues.<sup>6</sup> In addition the sulfonylureas have been shown to reduce the rate of glucose release from the liver<sup>7</sup> while the existence of a direct effect of insulin on hepatic glucose metabolism has been questioned.<sup>8</sup>

Although an increase in the peripheral arteriovenous (A-V) blood glucose concentration difference has usually been observed in human subjects following the intravenous administration of a single dose of 0.1 U insulin/kg or a total of 5 to 7 U,<sup>9-10</sup> doses of 3 U or less have frequently failed to produce

body glucose pool and suppressed the introduction of newly released glucose into the pool (glucogenesis) presumably from the liver. When insulin was given by the subcutaneous route in order to retard its rate of entrance into the circulating blood 10 U decreased the rate of glucogenesis without affecting glucose removal. A reduced net splanchnic output of glucose (NSGO) has been observed in man after either tolbutamide<sup>11-14</sup> or insulin.<sup>15</sup> These findings of various investigators suggested that in man small doses of insulin

\* The work reported in this paper was supported in part by the Diabetes Research Program of Western Reserve University through Research Grant A-1417 from the National Institute of Arthritis and Metabolic Diseases Public Health Service Bethesda MD and the Fund for the Study of Diabetes Mellitus and Related Metabolic Diseases at Western Reserve University.

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might have the same effects as the sulfonylureas on the NSGO and the peripheral uptake of glucose

This possibility has been tested by comparing the effects of tolbutamide and small doses of insulin on the NSGO and the simultaneously measured peripheral A-V blood glucose concentration difference in man

### Methods

The subjects for these studies were 25 nondiabetic hospital patients who were tested after an overnight fast. Eight of the subjects were tested with sodium tolbutamide,\* 11 received glucagon free crystalline insulin,† the other 6 served as control subjects and were given only 0.9 per cent sodium chloride solution. These substances were administered into a peripheral vein during a 2 min interval in the following doses: 2 gm of sodium tolbutamide either 2 or 3 U of insulin and 50 ml of sodium chloride solution. Blood samples were obtained simultaneously through indwelling needles in a radial artery and an antecubital vein and through a radiopaque catheter with its tip in a hepatic vein. Simultaneous arterial, peripheral venous and hepatic venous blood samples were obtained at 5 or 10 min intervals during a 15 to 35 min preliminary control period and for 50 min after injection of the test substance. The glucose content of the blood samples was determined in duplicate by the titrimetric method of Somogyi,<sup>16</sup> by this method a total of 23 determinations on 4 blood samples yielded a mean value of 85 mg/100 ml with a mean standard deviation of 2.1 mg/100 ml. The hepatic blood flow was estimated by the Bromsulphalein technique of Bradley *et al.*<sup>17</sup> with measurement of the plasma Bromsulphalein concentration.

the net output of glucose from the splanchnic system and a negative value would indicate a net uptake. The statistical significance of the differences between mean values was assessed by the *t* test of Fischer.

### Results

As seen in FIGURE 1, tolbutamide produced an average maximum arterial glucose concentration of 115.2 mg/100 ml in 30 min that was still maintained at a similar magnitude, with an average of 110.5 mg/100 ml at 50 min and a prompt return to the control value of 85.0 mg/100 ml. In the control group who were given sodium chloride no significant alteration in arterial blood glucose concentration was observed.

The average values for the HV-A blood glucose concentration difference are illustrated in FIGURE 2 and are summarized in TABLE 1. After tolbutamide administration the HV-A difference decreased in all 8 subjects. In 7 of the 8 subjects the average HV-A difference during the first 30 min after tolbutamide was at least 4 mg/100 ml lower than the average preliminary control

\* Supplied by C. J. O'Donovan of The Upjohn Company, Kalamazoo, Mich.

† Supplied by W. R. Kirtley of Eli Lilly and Company, Indianapolis, Ind.



FIGURE 1 Average changes in arterial blood glucose concentration produced by the peripheral intravenous administration of sodium tolbutamide (8 subjects), insulin (9 subjects), or sodium chloride (6 subjects)

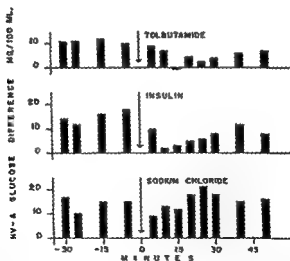


FIGURE 2 Average difference in blood glucose concentration before and after the peripheral intravenous administration of sodium tolbutamide (8 subjects), insulin (9 subjects), or sodium chloride (6 subjects). The first 4 bars on the left in each section represent values obtained during a preliminary control period.

TABLE 1

AVERAGE VALUES (+1 STANDARD DEVIATION) OF HV  $\Delta$  BLOOD GLUCOSE DIFFERENCE  
 NSGO PERIPHERAL  $\Delta$  BLOOD GLUCOSE DIFFERENCE AND ( $\Delta$  V)/ $\Delta$  RATIO IN  
 NONDIABETIC SUBJECTS BEFORE AND FOR THE FIRST 30 MIN AFTER THE  
 PERIPHERAL INTRAVENOUS ADMINISTRATION OF SODIUM TOLBUTAMIDE  
 INSULIN OR SODIUM CHLORIDE

Substance administered	No of subjects	HV $\Delta$ (mg /100 ml )		
		Before	After	p
Sodium tolbutamide	8	10.7 $\pm$ 6.5	4.1 $\pm$ 3.2	<0.05
Insulin	9	14.9 $\pm$ 7.6	5.7 $\pm$ 3.0	<0.01
Sodium chloride	6	14.1 $\pm$ 5.8	15.3 $\pm$ 10.4	>0.5
		NSGO (mg /min )		
		Before	After	p
Sodium tolbutamide	8	138 $\pm$ 59	50 $\pm$ 29	<0.01
Insulin	9	197 $\pm$ 101	73 $\pm$ 25	<0.01
Sodium chloride	6	176 $\pm$ 69	199 $\pm$ 120	>0.5
		$\Delta$ V (mg /100 ml )		
		Before	After	p
Sodium tolbutamide	6	2.2 $\pm$ 2.5	-1.0 $\pm$ 2.4	0.05
Insulin	9	2.9 $\pm$ 4.7	1.4 $\pm$ 3.8	>0.4
Sodium chloride	4	2.0 $\pm$ 1.7	3.0 $\pm$ 1.9	>0.4
		( $\Delta$ V)/ $\Delta$ ratio		
		Before	After	p
Sodium tolbutamide	6	0.30 $\pm$ 0.06	-0.21 $\pm$ 0.16	>0.05
Insulin	9	0.28 $\pm$ 0.14	0.19 $\pm$ 0.50	>0.5
Sodium chloride	4	0.23 $\pm$ 0.23	0.12 $\pm$ 0.25	>0.5

value. Similarly after insulin treatment there was a diminution in the HV A difference in 9 of the 11 subjects. In 8 of these 9 subjects the average decrease exceeded 4 mg/100 ml\*. After sodium chloride none of the control subjects showed a decline in the average HV A difference of as much as 4 mg/100 ml and there was no significant change in this difference for the control group as a whole.

The average values for the NSGO are illustrated in FIGURE 3 and are summarized in TABLE 1. Tolbutamide decreased the NSGO in all 8 subjects. The average value during the first 30 min after tolbutamide was at least 40 mg/min less than the preliminary control value in all 8 subjects. NSGO

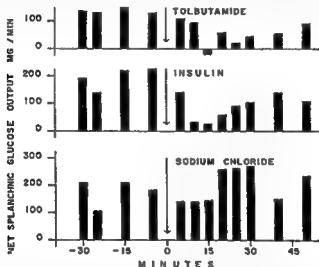


FIGURE 3. Average values for the NSGO before and after the peripheral intravenous administration of sodium tolbutamide (8 subjects), insulin (9 subjects) or sodium chloride (8 subjects). The first 4 bars on the left in each section represent values obtained during a preliminary control period.

decreased after insulin in 9 of the 11 subjects tested. In 8 of the 9 the decrease in the average NSGO exceeded 40 mg/min. After sodium chloride only

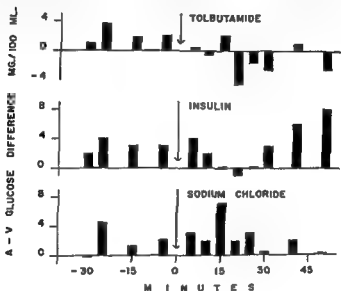
Although there were fluctuations in the estimated hepatic blood flow of individual subjects, none of the substances administered produced a significant change in the average flow.

The average values for the peripheral AV blood glucose concentration difference are illustrated in FIGURE 4 and are summarized in TABLE 1. No apparent increase in the AV difference was observed during the first 30 min.

\* Data from the two subjects in whom there was no demonstrable decrease in the HV A difference after insulin administration are not included in the figures or table.

after tolbutamide in 5 of the 6 subjects tested. In 3 of these 5 cases the average A V difference decreased more than 4 mg /100 ml and in 4 of them the values became negative. During the first 30 min after insulin there was no increase in the average A V difference in 6 of the 9 subjects, the increase

glucose toward the initial fasting level at 40 and 50 min after insulin administration was accompanied by an increased peripheral A V difference that was



section represent values obtained during a preliminary control period

at least 4 mg / 100 ml greater than the preliminary control value in 5 of the 9 subjects. None of the 4 control subjects tested with sodium chloride showed an increase in A V difference as great as 4 mg /100 ml and there was no significant change in the A V difference for the group as a whole.

that was accompanied by a decrease in NSGU without an increase in peripheral A V blood glucose concentration difference demonstrable by the techniques employed

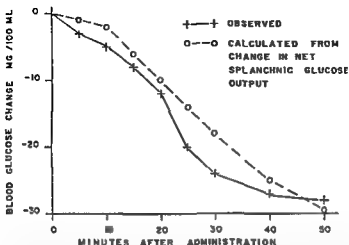


FIGURE 5 A comparison of the average arterial blood glucose concentration changes after tolbutamide administration with the calculated changes that would be produced by the observed alterations in NSGO. The assumptions involved in this calculation are discussed in the text.

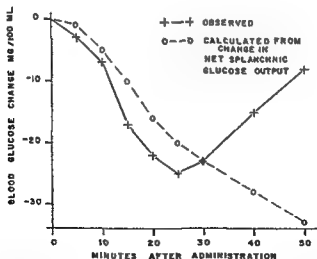


FIGURE 6 A comparison of the average arterial blood glucose concentration changes after insulin administration with the calculated changes that would be produced by the observed alterations in NSGO. The assumptions involved in this calculation are discussed in the text.

## DISCUSSION

The techniques employed in this study impose certain limitations on the conclusions that may be drawn from the results obtained.

(1) Since the  $A-V$  glucose differences were measured only across the splanchnic bed and in an upper extremity changes in glucose assimilation in other organs would not be detected.

(2) A decrease in  $VSGO$  could result from a diminution in the net hepatic glucose output  $\equiv$  an increase in the glucose uptake in an extrahepatic splanchnic component such as adipose tissue or both. The present results do not allow a distinction among these possibilities and do not permit a decision as to whether tolbutamide and insulin act at the same or different sites within the splanchnic system. Studies with  $C^{14}$  labeled glucose in animals<sup>7, 20, 21</sup> and man<sup>12, 22</sup> have shown that sulfonylurea induced hypoglycemia  $\equiv$  accompanied by an inhibition of glucogenesis presumably from the liver. From similar studies with insulin some investigators<sup>12, 20, 23</sup> have concluded that one action of the hormone was a reduction of the rate of glucogenesis while others<sup>7, 24</sup> have been unable to demonstrate such an effect consistently. Direct measurements of the hepatic venous portal venous glucose difference in the dog have shown a decrease in net hepatic glucose output after tolbutamide, but not after insulin administration.<sup>7</sup> Such direct measurements are not available in man.

(3) The limitations of the analytical method for glucose and the necessity for assuming the constancy of the peripheral blood flow make the measurement of the  $A-V$  glucose difference a relatively insensitive technique for the detection of small changes in peripheral glucose uptake. The failure to detect an increment in  $A-V$  glucose difference by this technique does not exclude the occurrence of small increases in this difference and therefore should not be considered to prove that neither tolbutamide nor insulin in this dosage exerts any peripheral effect. On the other hand if changes as large as those observed in the splanchnic system occurred in skeletal muscle and skin there is no reason to believe that they would escape detection since the total blood flow to resting skeletal muscle and skin in man is similar in magnitude to the splanchnic blood flow.<sup>25, 26</sup>

(4) The present studies do not indicate the mechanism of action of tolbutamide and insulin. The two hypoglycemic agents might have different and independent mechanisms of action even though their sites of action were the same.

(5) In man the sites of action of endogenous and exogenous insulin might differ. Such a discrepancy might be due to differences in their loci of entry into the blood stream<sup>27</sup> alterations produced in the exogenous hormone during its preparation<sup>28</sup> or the heterologous nature of exogenous insulin from animal sources.<sup>29</sup>

It is of interest to determine whether the observed decrease in  $VSGO$  is adequate to account for the fall in the arterial blood glucose concentration that followed tolbutamide or insulin administration. If it is assumed that the rate of glucose uptake by the non-splanchnic tissues remained constant

and that glucose was distributed uniformly in the extracellular fluid volume, estimated as 20 per cent of the body weight,<sup>21</sup> the decline in the blood glucose concentration that would result from the observed decrease in NSGO can be calculated. The results of these calculations are shown in FIGURES 5 and 6. Although there are large discrepancies between the predicted and observed blood glucose decreases in individual cases, the average values are similar in magnitude after both tolbutamide and insulin. It is concluded that the decrease in NSGO could be the major cause of the blood glucose fall after tolbutamide or insulin, but the occurrence of an increased glucose uptake in a non-planchnic tissue cannot be excluded. In these studies the return of the blood glucose concentration toward the initial fasting level after insulin induced hypoglycemia is not adequately accounted for by the observed changes in NSGO or peripheral A-V glucose difference.

The similarity of the results obtained with tolbutamide and small doses of insulin appears compatible with an insulin-mediated hypoglycemic action of the sulfonylureas. However, because of the limitations of the techniques employed, other mechanisms of action are not excluded.

### Summary

In nondiabetic human subjects the arterial blood glucose decline produced by the peripheral intravenous administration of tolbutamide was accompanied by a reduction in NSGO sufficient to account for the major portion of the blood glucose fall. No increase in the peripheral A-V glucose difference was demonstrated.

Similar changes were produced by small doses of insulin.

The similarity of the effects produced by tolbutamide and insulin is compatible with an insulin-mediated hypoglycemic action of the sulfonylureas. This conclusion must be tempered by recognition of the limitations of the experimental techniques employed.

### Acknowledgments

We express our appreciation to T. W. Moir, A. B. Lord, and W. B. Newberry, Jr., who performed the hepatic vein catheterizations, and to Nancy Hofmann, Bryant Kingsbury, and Frank Mills, who gave valuable technical assistance.

### References

- 1 LOUBATIÈRES A. 1957. The mechanism of action of the hypoglycemic sulfonamides. A concept based on investigations in animals and in man. *Diabetes* 6: 408-417.
- 2 MIRSKY I. A. G. PERINETTI & S. GITELSON. 1957. The role of insulinase in the hypoglycemic response to sulfonylureas. *Ann. N. Y. Acad. Sci.* 71(1): 103-111.
- 3 POZZA G. G. GALANINO & P. P. FOX. 1956. Insulin secretion following carbutoamide injections in normal dogs. *Proc. Soc. Exptl. Biol. Med.* 93: 539-542.
- 4 LOUBATIÈRES A. 1946. Etude physiologique et pharmacodynamique de certains dérivés sulfamidés hypoglycémisants. *Arch. Intern. Physiol.* 54: 174-177.
- 5 GOETZ I. C. & R. H. LEIDAL. 1958. Direct demonstration of insulin release from pancreas by tolbutamide. *Federation Proc.* 17: 55.



- 6 STADIE W C 1958 Is the metabolism of peripheral tissues affected by the aryl sulfonylureas? *Diabetes* 7 63-65
- 7 ASHMORE J G F CARILL JR A S EARLE & S ZOTTU 1958 Studies on the disposition of blood glucose. A comparison of insulin and Orinase *Diabetes* 7 1-8
- 8 LEVINE M & I B FRITZ 1956 The relation of insulin to liver metabolism *Diabetes* 5 209-219
- 9 SOMOGYI M 1949 Studies of arteriovenous differences in blood sugar. III Effect of insulin administered intravenously in the postabsorptive state *J Biol Chem* 179 237-238
- 10 GOETZ F C A S GILBERTSEN & V JOSEPHSON 1956 Acute effects of Orinase on peripheral glucose utilization *Metabolism* 5 788-800
- 11 BELL D M & T BRUNS 1952 Effect on femoral and glucose difference of insulin injected into an antecubital vein and into a femoral artery *J Clin Invest* 31 717-720
- 12 REICANT L & G L FISCHER 1957 Studies on the mechanism of tolbutamide hypoglycemia in animal and human subjects *Ann N Y Acad Sci* 71(1) 62-70
- 13 JACOBS G G REICHARD E H GOODMAN JR M FRIEDMAN & S WEINHOFF 1958 Action of insulin and tolbutamide on blood glucose entry and removal *Diabetes* 7 358-364
- 14 KIBLER R F & G GORDON 1956 Effect of tolbutamides (Orinase) on net splanchnic glucose production (NSGP) in man (abstract) *J Lab Clin Med* 48 824
- 15 BEAR A G B H BILLING & S SHERLOCK 1952 The response of the liver to insulin in normal subjects and in diabetes mellitus. hepatic vein catheterisation studies *Clin Sci* 11 151-165
- 16 SOMOGYI M 1945 Determination of blood sugar *J Biol Chem* 160 69-73
- 17 BRADLEY S E F J ENGELINGER G P BRADLEY & J J CURRY 1945 The estimation of hepatic blood flow in man *J Clin Invest* 24 890-897
- 18 GAEBLER O H 1945 Determination of Bromsulphalein in normal turbid hemolyzed or icteric serums *Am J Clin Pathol* 16 452-455
- 19 SOMOGYI M 1948 Studies of arteriovenous differences in blood sugar. I Effect of alimentary hyperglycemia on the rate of extrahepatic glucose assimilation *J Biol Chem* 174 189-200
- 20 BERSON S A & R S ALOW 1957 Some remarks on the mechanism of action of the sulfonylureas *Diabetes* 6 274-277
- 21 TARDING P & P SCHAMBY 1958 The action of sulfonylureas and insulin on the glucose output from the liver of normal dogs *Endocrinologie* 35 222-225
- 22 SEARLE G L C F MORTIMORE R BUTCHLEY & W A REILLY 1958 The influence of insulin and Orinase on the turnover of plasma glucose as studied with C<sup>14</sup> glucose in humans (abstract) *Clin Research* 6 92
- 23 HENDERSON M J C A WERNSHALL & P ODENSE 1955 Effects of insulin on rates of glucose transfer in the depancreatized dog *Can J Biochem Physiol* 33 926-939
- 24 DUNN D F B FRIEDMAN A R MAAS G A REICHARD & S WEINHOFF 1957 Effects of insulin on blood glucose entry and removal rates in normal dogs *J Biol Chem* 225 225-237
- 25 WALL J S R STEELE R C DE BODO & A ALTSZILLER 1957 Effect of insulin on utilization and production of circulating glucose *Am J Physiol* 189 43-50
- 26 COOPER K E O G EDHOLM & R F MORTIM 1955 The blood flow in skin and muscle of the human forearm *J Physiol* 128 258-267
- 27 ANDRES R G CADEN & K I ZIERLER 1956 The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm *J Clin Invest* 35 671-682
- 28 MADISON L L & R H UNGER 1958 The physiologic significance of the secretion of endogenous insulin into the portal circulation. I Comparison of the effects of glucagon free insulin administered via the portal vein and via a peripheral vein on

- the magnitude of hypoglycemia and peripheral glucose utilization. *J Clin Invest* **37**: 631-639
29. MOLONEY P J & M COVAL. 1955. Antigemcity of insulin diabetes induced by specific antilodies. *Biochem J* **59**: 179-185
30. BROWN H T, SANGER & R KITAI. 1955. The structure of pig and sheep insulins. *Biochem J* **60**: 556-565
31. SHREEVE W W, N BAKER, M MILLER, R A SHIPLEY, G I INCEPY & J W CRAIG. 1956.  $C^{14}$  studies in carbohydrate metabolism. II. The oxidation of glucose in diabetic human subjects. *Metabolism* **5**: 22-34

# THE RELATIONSHIP BETWEEN THE MECHANISM OF ACTION OF THE SULFONYLUREAS AND THE SECRETION OF INSULIN INTO THE PORTAL CIRCULATION\*

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The conclusion that the hypoglycemic effect of the sulfonylureas is mediated through the secretion of endogenous insulin is based upon data indicating that the presence of functional pancreatic beta cells is prerequisite for their hypoglycemic action. In all experimental preparations in which the beta cells are ablated including completely alloxanized, depancreatized, or eviscerated animals the sulfonylureas do not significantly lower the blood sugar.<sup>1,4</sup> By contrast hypoglycemia eventuates whenever the beta cells are intact,<sup>5</sup> even in hepatectomized animals.<sup>6,7</sup> Recently Pfeiffer has reported an increase in insulinlike activity of the portal venous blood after sulfonyl urea administration.<sup>8</sup>

Despite the formidable evidence linking the hypoglycemic effect with the presence of beta cells objections to the insulinogenic mechanism of action have been raised. For the most part these objections are based upon the failure of the sulfonylureas to duplicate precisely the metabolic effects of exogenous insulin on a variety of parameters. The sulfonylureas are said to produce hypoglycemia by decreasing the hepatic output of glucose.<sup>9,10</sup> This hepatic effect and the failure of the sulfonylureas to increase peripheral glucose utilization consistently<sup>11,12</sup> have been cited as physiological actions inconsistent with the beta-cytotropic mechanism of action. It should be pointed out that such objections implicitly assume that endogenous insulin reduces blood glucose concentration primarily by increasing peripheral glucose utilization and has little or no effect on the hepatic output of glucose.

To conclude from these conflicting data that the sulfonylureas do not stimulate endogenous insulin secretion leaves their dependence on intact beta cells obscure and unexplained. In view of this beta cell dependence, an alternative conclusion would be that the sulfonylureas stimulate the secretion of endogenous insulin which, in turn, may differ in its physiological effects from exogenous insulin. The natural hormone and its commercial counterpart exogenous insulin may differ for several reasons: (1) exogenous insulin may be altered in its commercial preparation, (2) exogenous insulin prepared from one species and administered to another species may not have physiological effects identical to the species specific endogenous hormone, (3) the metabolic effect of exogenous insulin may be altered because it enters the body through the systemic circulation. Under physiological conditions endogenous insulin is secreted into branches of the portal circulation, there

\* The work described in this paper was supported in part by grants from The Upjohn Company, Kalamazoo, Mich. and The Dallas Heart Association, Dallas, Texas.

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fore, all of the natural hormone must traverse the liver before entry into the systemic circulation, whence it becomes available to the peripheral tissues.

The delivery of endogenous insulin to the liver in amounts and concentration greater than those possible for any other tissue in the body suggests that the secretion into the portal circulation has physiological significance. Nevertheless the controversy as to whether insulin has a direct effect on the hepatic output of glucose still persists.<sup>11-16</sup> *In vitro* effects of insulin on the liver have been difficult to demonstrate but *in vivo* effects of varying duration and magnitude have been reported.<sup>17-21</sup> Recently new evidence both *in vitro*<sup>24</sup> and *in vivo*<sup>20,22</sup> supporting a direct effect of insulin on hepatic glucose metabolism has appeared. The data of Wall,<sup>23</sup> Dunn,<sup>24</sup> Jacobs<sup>25</sup> and their colleagues obtained by the technique of determining changes in the specific

intravenously there results only a transient decrease in hepatic glucose output and a prolonged increase in peripheral glucose utilization of considerable magnitude. By contrast when insulin is administered subcutaneously or by slow intravenous infusion a depression of hepatic glucose output of considerable duration ensues. In the present studies different parameters, the arteriovenous (A-V) glucose difference across the leg<sup>26</sup> and the hepatic output of glucose,<sup>27</sup>

subjects during the intravenous infusion of glucagon free insulin administered for 120 min at a rate of 0.06 to 0.1 U/min. The changes in A-V glucose difference were then compared to changes that occurred in the same 8 subjects following the intravenous administration of 1 gm of sodium tolbutamide. Femoral arterial and venous blood samples were collected simultaneously at 10 min intervals during a 140- to 180 min period by means of indwelling Cournand needles. Blood glucose concentrations were determined by the Somogyi copper iodometric method<sup>28</sup> on 2 ml blood samples.

During insulin infusion mean arterial blood glucose concentration fell from 80 to 52 mg per cent, femoral A-V glucose difference failed to increase and averaged only 1.8 mg per cent. Similar results occurred after tolbutamide administration. Mean blood glucose concentration fell from 81 to 52 mg per cent and A-V glucose differences averaged 2.3 mg per cent. The data indicate no significant difference in peripheral glucose utilization as manifest by changes in A-V glucose difference after tolbutamide and after the slow infusion of insulin. While it is possible that peripheral glucose utilization was increased but could not be detected after insulin administration, these data can be alternately interpreted to indicate an hepatic effect of both tolbutamide and insulin under these experimental conditions. Such an interpretation is in accord with the previously mentioned data of Jacobs *et al* who noted an hepatic effect of insulin administered subcutaneously or by slow intravenous infusion.<sup>22</sup> A typical set of experiments showing the effects on A-V glucose difference of insulin and tolbutamide are shown in FIGURES 1 and 2.

# THE RELATIONSHIP BETWEEN THE MECHANISM OF ACTION OF THE SULFONYLUREAS AND THE SECRETION OF INSULIN INTO THE PORTAL CIRCULATION\*

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The conclusion that the hypoglycemic effect of the sulfonylureas is mediated through the secretion of endogenous insulin is based upon data indicating that the presence of functional pancreatic beta cells is prerequisite for their hypoglycemic action. In all experimental preparations in which the beta cells are ablated including completely alloxanized, depancreatized or eviscerated animals, the sulfonylureas do not significantly lower the blood sugar.<sup>1-4</sup> By contrast, hypoglycemia eventuates whenever the beta cells are intact,<sup>5</sup> even in hepatectomized animals.<sup>6-7</sup> Recently Pfeiffer has reported an increase in insulinlike activity of the portal venous blood after sulfonylurea administration.<sup>8</sup>

Despite the formidable evidence linking the hypoglycemic effect with the presence of beta cells, objections to the insulinogenic mechanism of action have been raised. For the most part these objections are based upon the failure of the sulfonylureas to duplicate precisely the metabolic effects of exogenous insulin on a variety of parameters. The sulfonylureas are said to produce hypoglycemia by decreasing the hepatic output of glucose.<sup>9,10</sup> This hepatic effect and the failure of the sulfonylureas to increase peripheral glucose utilization consistently<sup>12-16</sup> have been cited as physiological actions inconsistent with the beta-cytotropic mechanism of action. It should be pointed out that such objections implicitly assume that endogenous insulin reduces blood glucose concentration primarily by increasing peripheral glucose utilization and has little or no effect on the hepatic output of glucose.

To conclude from these conflicting data that the sulfonylureas do not stimulate endogenous insulin secretion leaves their dependence on intact beta cells obscure and unexplained. In view of this beta-cell dependence, an alternative conclusion would be that the sulfonylureas stimulate the

physiological  
 its commercial  
 (1) exogenous

insulin may be altered in its commercial preparation (2) exogenous insulin

the body through the systemic circulation. Under physiological conditions endogenous insulin is secreted into branches of the portal circulation, there

\* The work described in this paper was supported in part by grants from The Upjohn Company, Kalamazoo, Mich. and The Dallas Heart Association, Dallas, Texas.  
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fore all of the natural hormone must traverse the liver before entry into the systemic circulation whence it becomes available to the peripheral tissues.

The delivery of endogenous insulin to the liver in amounts and concentration greater than those possible for any other tissue in the body suggests that the secretion into the portal circulation has physiological significance. Nevertheless the controversy as to whether insulin has a direct effect on the hepatic output of glucose still persists.<sup>11-16</sup> *In vitro* effects of insulin on the liver have been difficult to demonstrate but *in vivo* effects of varying duration and magnitude have been reported.<sup>17-19</sup> Recently new evidence both *in vitro*<sup>11</sup> and *in vivo*<sup>20-23</sup> supporting a direct effect of insulin on hepatic glucose metabolism has appeared. The data of Wall,<sup>20</sup> Dunn,<sup>21</sup> Jacobs<sup>22</sup> and their colleagues obtained by the technique of determining changes in the specific activity of a trace dose of uniformly labeled glucose C<sup>14</sup> after insulin administration indicate that the rate of insulin administration appears to affect the magnitude of the hepatic response. When insulin in large amounts is injected intravenously there results only a transient decrease in hepatic glucose output and a prolonged increase in peripheral glucose utilization of considerable magnitude. By contrast when insulin is administered subcutaneously or by slow intravenous infusion a depression of hepatic glucose output of considerable duration ensues. In the present studies different parameters the arteriovenous (A-V) glucose difference across the leg<sup>24</sup> and the hepatic output of glucose<sup>25</sup> were measured during the slow infusion of glucagon free insulin and a similar depression of hepatic glucose output was found.

Changes in femoral A-V glucose difference were determined in 8 normal subjects during the intravenous infusion of glucagon free insulin administered for 120 min at a rate of 0.06 to 0.1 U/min. The changes in A-V glucose difference were then compared to changes that occurred in the same 8 subjects following the intravenous administration of 1 gm of sodium tolbutamide. Femoral arterial and venous blood samples were collected simultaneously at 10 min intervals during a 150- to 180-min period by means of indwelling Courmand needles. Blood glucose concentrations were determined by the Somogyi-copper iodometric method<sup>26</sup> on 2 ml blood samples.

During insulin infusion mean arterial blood glucose concentration fell from 80 to 52 mg per cent, femoral A-V glucose difference failed to increase and averaged only 1.8 mg per cent. Similar results occurred after tolbutamide administration. Mean blood glucose concentration fell from 93 to 52 mg per cent and A-V glucose differences averaged 2.1 mg per cent. The data indicate no significant difference in peripheral glucose utilization as manifest by changes in A-V glucose difference after tolbutamide and after the slow infusion of insulin. While it is possible that peripheral glucose utilization was increased but could not be detected after insulin administration these data can be alternately interpreted to indicate an hepatic effect of both tolbutamide and insulin under these experimental conditions. Such an interpretation is in accord with the previously mentioned data of Jacobs *et al* who noted an hepatic effect of insulin administered subcutaneously or by slow intravenous infusion.<sup>22</sup> A typical set of experiments showing the effects on A-V glucose difference of insulin and tolbutamide are shown in FIGURES 1 and 2.

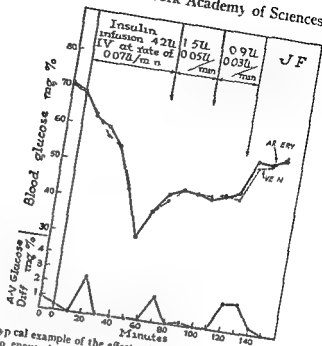


FIGURE 1 Typical example of the effects of the slow infusion of glucagon free insulin on femoral arteriovenous glucose difference in a normal subject

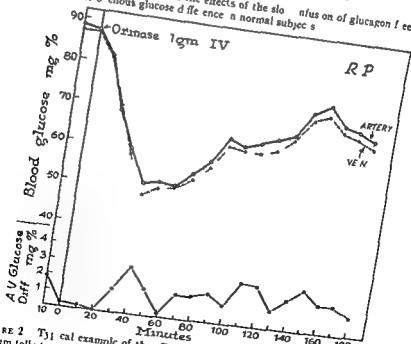


FIGURE 2 Typical example of the effects of the intravenous administration of 1 gm of sodium tolutamide on the femoral arteriovenous glucose difference in a normal

In order to determine by direct measurement whether the slow infusion of insulin results in a decreased hepatic output of glucose hepatic blood flow and hepatic vein arterial glucose differences were measured in normal and diabetic subjects. Following hepatic vein catheterization hepatic blood flow was measured by the Bromsulphalein technique of Bradley *et al*.<sup>22</sup> Simultaneous hepatic venous and arterial blood samples were collected at 10-min intervals for 20 to 30 min prior to and during the 60 min duration of the insulin infusion. Examples of two experiments one in a diabetic subject and the other in a normal subject are shown in TABLE 1 and in FIGURE 3. In both instances the hypoglycemia following the insulin infusion was associated with a profound reduction in the hepatic output of glucose.

TABLE 1

EFFECT OF INSULIN INFUSION ON HEPATIC GLUCOSE OUTPUT IN A LABILE DIABETIC

Time (minutes)	Estimated hepatic blood flow (ml/min)	Blood glucose (mg/100 ml)			Hepatic glucose output (mg/min)
		Hepatic vein	Femoral artery	IV Art	
-24	962	450	270	180	1730
-15	1144	415	269	206	2160
-5	1177	427	274	151	1600
	Start insulin infusion 0.1 U/min				
10	1030	374	275	99	1010
20	1121	335	258	117	1540
30	970	345	250	99	922
40	923	301	240	71	660
50	907	311	221	90	820
60	895	210	207	3	27
70	893	210	185	25	222

In order to obviate the criticism that these changes reflect only alterations in net splanchnic glucose output and not necessarily in net hepatic glucose output eight experiments were performed on dogs with complete portacaval shunts. In this preparation all the blood perfusing the liver comes from the arterial circulation. A typical example of the effect of the infusion of 0.025 U/min of glucagon free insulin on hepatic venous and arterial blood glucose concentrations and on the hepatic output of glucose is shown in FIGURE 4.

These data indicate that under proper experimental conditions exogenous insulin apparently has an effect upon hepatic glucose metabolism. Since all endogenous insulin must traverse the liver before entering the systemic circulation the concentration and amount that reach the liver must exceed those reaching all other tissues. This suggests that endogenous insulin



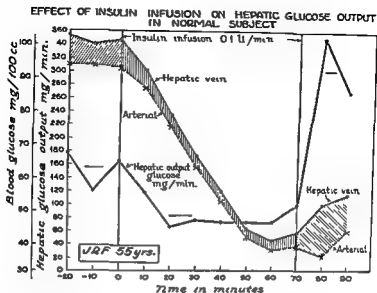


FIGURE 3 Effect of the slow infusion (0.1 U/min) of glucagon free insulin on the hepatic venous arterial glucose difference and estimated hepatic output of glucose in a normal subject

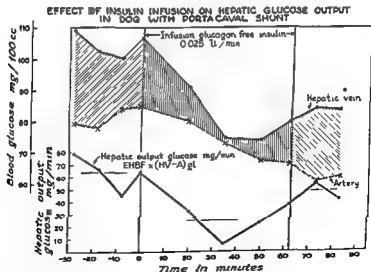


FIGURE 4 Effect of the slow infusion (0.025 U/min) of glucagon free insulin on the hepatic venous arterial glucose difference and hepatic output of glucose in a dog with a complete portacaval shunt

may have a greater hepatic effect than insulin administered into the systemic circulation and therefore may account for the hepatic effect of the sulfonylureas.

In order to determine whether the initial passage of insulin through the liver alters the magnitude of its hepatic and peripheral actions, the effects of glucagon free insulin administered by the portal vein and by a peripheral vein were compared in 16 experiments on 8 dogs. The details of these experiments have already been reported.<sup>28</sup> In these studies the magnitude of the arterial hypoglycemia was the same after both routes of administration. However in each dog the peripheral injection of insulin resulted in a significantly greater increase in peripheral glucose utilization than did the portal administration. The combination of similar arterial hypoglycemia coupled with a lesser augmentation of peripheral glucose utilization after portal injection suggests that a substantial amount of insulin is retained by the

liver than when injected into the inferior vena cava.<sup>29</sup> A typical example of the comparative effects of the portal and peripheral routes of insulin administration on the magnitude of arterial hypoglycemia and on the magnitude of increase in peripheral glucose utilization is shown in FIGURE 5.

The apparently greater hepatic effect of insulin injected by the portal vein suggests that a substantial amount of this insulin is retained by the liver during the initial transhepatic passage. Studies were designed to determine whether a significant amount of insulin is retained by the liver during a single circulation and also to compare the peak hepatic binding of insulin after portal and peripheral administration.<sup>30</sup> In the first study 196 rats were injected with  $I^{131}$  labeled insulin. In one half of the rats the insulin (0.002 units) was injected into the portal vein, in the other one half into the femoral vein. The rats were sacrificed at frequent intervals from 4 sec to 15 min after insulin injection. Immediately thereafter the liver was perfused with 30 to 50 cc of modified Ringer's solution. The liver, kidneys and other tissues were then assayed for radioactivity in a well type gamma counter and the percentage of the administered dose bound in each organ was determined. The results indicate that during the single transhepatic circulation following portal injection 51 per cent of the administered insulin was bound firmly to the rat liver. By contrast peak hepatic binding after the peripheral route of administration was only 27 per cent of the administered dose.

In order to determine whether the human liver binds a significant amount of insulin during a single transhepatic circulation, the following study was performed in 12 human subjects without known hepatic or pancreatic disease.<sup>31</sup> During abdominal operation a solution containing 0.15  $\mu$ l of  $I^{131}$  labeled insulin and a known amount of insulin was injected rapidly into the portal vein. Ten seconds later a brachial arterial blood sample was collected over a 10 to 15 sec period. The concentrations of insulin and insulin  $I^{131}$  were then determined. Since known amounts of insulin  $I^{131}$  and insulin were

injected, their volumes of distribution could be calculated and compared. Control studies were performed in 8 subjects by injecting the solution of inulin and insulin  $I^{131}$  into the antecubital vein and collecting the arterial blood sample at precisely the same time as after portal injection.

In the control studies the volumes of distribution of labeled insulin (2038 ml) and inulin (1989 ml) were almost identical. By contrast, after portal injection the volume of distribution of the labeled insulin (5581 ml) was more than twice as large as that of inulin (2584 ml). This indicates that 52 per cent of the insulin was bound to the human liver in a single transhepatic circulation.

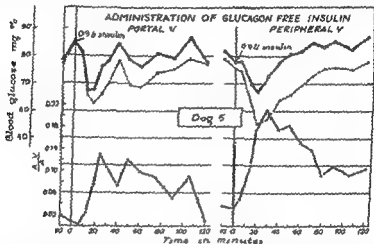


FIGURE 5. Typical example of the comparative effects of glucagon free insulin administered by the portal vein and by a peripheral vein on the magnitude of the hypoglycemia and the magnitude of the increase in peripheral arteriovenous glucose difference. Note that the degree of hypoglycemia is the same after both routes of administration and that the arteriovenous glucose differences are greater after the peripheral administration of insulin. Reproduced by permission of *The Journal of Clinical Investigation*.<sup>20</sup>

These data support but do not prove the concept that endogenous insulin in man regulates the hepatic output of glucose. However, they should not be construed to mean that this is the sole function of insulin. We have other data indicating that, when the hepatic insulin binding sites are saturated<sup>22</sup> or blocked,<sup>23</sup> a greater portion of endogenous insulin passes through the liver to the periphery where it exerts a greater effect on peripheral glucose utilization.<sup>24</sup> Crandall and Cherry have also previously reported that changes in the hepatic output of glucose follow anesthesia and acute operative stress.<sup>25</sup>

#### Summary and Conclusions

There is a growing body of evidence not yet incontrovertible, indicating that insulin, when administered under proper experimental conditions, results in a decreased hepatic glucose output and thereby contributes to the





# CHANGES INDUCED BY INSULIN AND TOLBUTAMIDE IN THE GLUCOSE OUTPUT OF THE LIVER

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Following the independent observation of the additional hypoglycemic effect of the sulfonylureas in insufficiently insulin treated pancreatectomized dogs<sup>1</sup> it became evident that these compounds might produce the decrease in the blood sugar concentration of both normal and diabetic dogs by inhibiting either partly or totally the release of glucose from the liver.

The first part of this report describes our experimental observations after applying the single injection of C<sup>14</sup> labeled glucose for measuring the glucose output in the liver in normal dogs before and after intravenous injections of either tolbutamide or insulin.

A new method for calculating this output from the isotope data was developed. By equating the ratio between specific activity of glucose in samples of hepatic venous and arterial blood to the amount of glucose entering and leaving the liver, the output of unlabeled glucose from this organ could be calculated for any period before or after the administration of a substance producing a change in the size of the glucose pool. This method may have an advantage over the ordinary isotope dilution method based upon injection of C<sup>14</sup> glucose, which does not allow calculation of the glucose turnover during the actual changes of the plasma glucose concentration as the latter will not necessarily be representative of the various compartments of the glucose pool.

The second part of the report deals with the results obtained with this method when insulin was infused partially into normal cyclopropane anesthetized dogs.

## *Effect of Intravenous Injections of Tolbutamide or Insulin\**

These studies were performed in normal dogs fasted overnight and anesthetized with pentobarbital sodium (Nembutal) a dose of about 200  $\mu$ g of uniformly labeled C<sup>14</sup> glucose (Amersham Pharmacia) injected intravenously. Blood samples were collected in a peripheral vein. After a control period of about 1 hour a total dose of

insulin 2 doses (0.10 gm/kg) samples were collected at

As shown in FIGURE 1

the exponential decrease of the blood glucose specific activity observed in the control period, which reflected a constant release of unlabeled glucose from the liver to replace the amount of glucose utilized in the peripheral tissues was interrupted by the injection of the tolbutamide as the specific activity remained almost constant for a period of 30 to 40 min. During this time the plasma glucose concentration decreased to about 40 to 50 mg per cent. When these hypoglycemic levels were reached, the specific activity

again decreased exponentially at a rate slightly higher than in the control period but nevertheless corresponding to a lesser release of glucose from the liver

For the example shown in FIGURE 1 the rate of glucose release from the liver was about 57 mg/min in the control period. Assuming that a similar

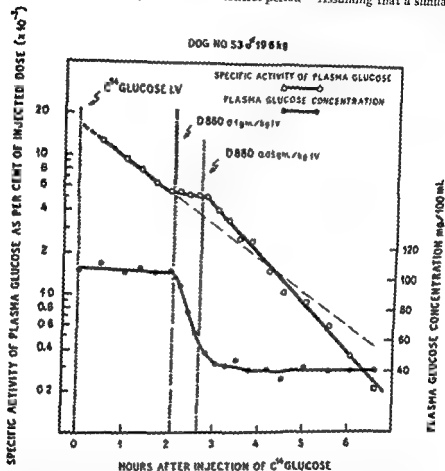


FIGURE 1 The effect of intravenously injected tolbutamide (D 860) on the plasma glucose concentration and specific activity in an anesthetized normal dog

rate of peripheral glucose utilization persists under the actual decrease in the blood sugar, this rate would be sufficient to decrease the blood sugar to the level observed, as this would require a utilization of about 65 mg/min, which is not significantly different from the control value. The steeper decline later in the postinjection period, indicating the rate to be about 45 mg/min, does not reflect an increased rate of utilization, but is due to the considerably smaller glucose pool.

Searle and Chaikoff<sup>3</sup> observed a similar plateau after creating a hyper

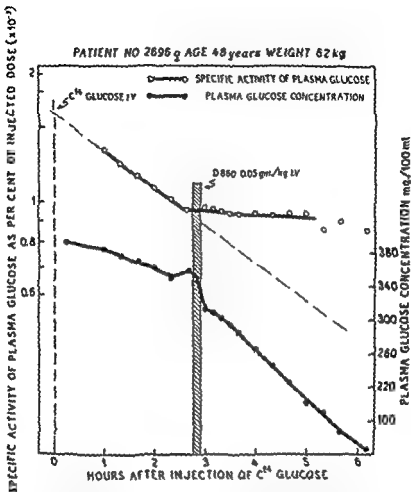


FIGURE 2 The effect of intravenously injected tolbutamide (D 860) on the plasma glucose concentration and specific activity in an unanesthetized diabetic patient who received the last injection of regular insulin 18 hours prior to the examination

glycemia that was interpreted as an inhibition of the glucose output. According to this, the practically constant specific activity of the blood glucose after the injection of tolbutamide may also indicate a block in the glucose release from the liver. The short duration of a complete inhibition is thought to be due to the measures taken by the organism to overcome the large degree of hypoglycemia shortly after it has been established.

Actually this explanation was indirectly confirmed when a plateau in the specific activity curve was observed under identical experimental conditions.



in 2 diabetic patients and 2 pancreatectomized dogs. In these cases the plateau lasted for the entire postinjection period of observation (FIGURES 2 and 3). Although exogenous insulin (20 IU lente insulin) had been administered to the diabetic dogs only 18 hours before the injection of tolbutamide a very clear cut inhibition of the glucose output of the liver was produced (FIGURE 3). As a result of this the blood sugar decreased from about 550 mg per cent to about 250 mg per cent in the course of 4 hours. This decrease was produced by the rate of glucose uptake in the peripheral tissues and the excretion in the urine.

The experimental data of Creutzfeldt<sup>4</sup> and others indicate that the hypoglycemia might be produced by a stimulation of the release of insulin from the beta cells of the Langerhans islets. Therefore identical experiments were carried out replacing tolbutamide with 2 intravenous injections of

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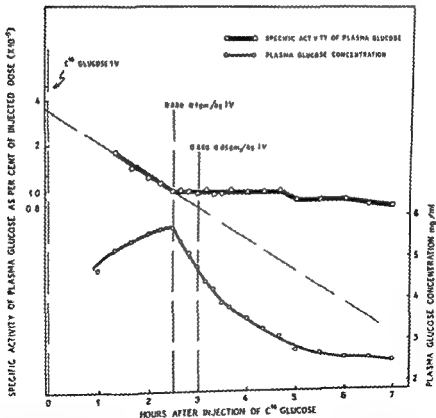


FIGURE 3 The effect of intravenously injected tolbutamide (U 860) on the plasma glucose concentration and specific activity in an anesthetized pancreatectomized dog which 18 hours before the injection of the  $C^{14}$  glucose received its last dose of insulin (lente insulin 14 IU + insulin 6 IU).

inulin (0.1 IU and 0.05 IU/kg at 10 min intervals). In contrast to the findings after tolbutamide this was immediately followed by a considerably steeper decrease in the specific activity which cannot be produced by an inhibition of the glucose release from the liver (FIGURE 4).

In an independent study Dunn *et al.*<sup>8</sup> observed a very short plateau (5 to 10 min) in the specific activity curve following doses of insulin in the range

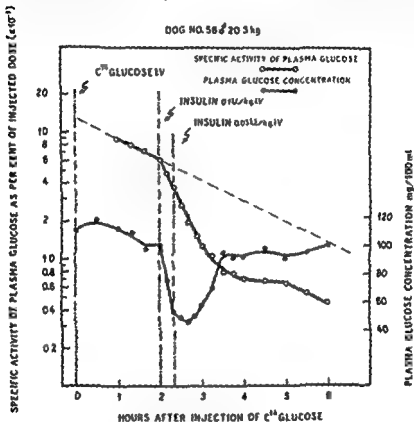


FIGURE 4 The effect of intravenously injected insulin on the plasma glucose concentration and specific activity in an anesthetized normal dog.

of 1 IU/kg. To a certain extent we can confirm this observation, as doses of insulin exceeding 0.5 IU/kg produce what appears to be a very short lasting plateau indeed far shorter than that observed after tolbutamide. This may be related to these rather unphysiological doses of insulin especially as it is not observed in our experiments when smaller and perhaps more physiological doses of insulin are injected. Therefore from our point of view it is not likely that the hypoglycemic effect of tolbutamide is due to an enhancement of the insulin release from the pancreas.

However, in order to test the possibility that an increase of the insulin secreted from the pancreas into the portal blood might have a special and limited effect upon the glucose output from the liver, the subsequent study of the effect of insulin constantly infused intraportally in amounts varying from 0.02 to 0.12 I U/kg 'min was undertaken.

*The Glucose Output of the Liver Following Intraportal Insulin Infusions*

The principles of the calculation of the amount of unlabeled glucose released from the liver are shown schematically in FIGURE 5. The specific activity

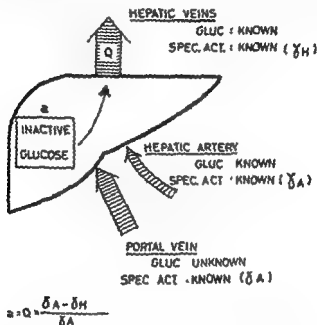


FIGURE 5 The principle of the isotope dilution method worked out for calculating the release of unlabeled glucose (a) from the liver ( $Q$  = total amount of glucose leaving the liver)

of the glucose in portal and arterial blood is identical if no dilution occurs with unlabeled glucose absorbed from the intestines. This is confirmed experimentally in normal dogs starved for 18 hours, although the concentration of glucose may differ in blood from these two vessels. The arterial specific activity can thus be regarded as representative for all blood glucose entering the liver. If  $\gamma_A$  and  $\gamma_H$  represent the specific activity of glucose in arterial and hepatic venous blood, the ratio  $\gamma_A / \gamma_H$  will be equal to the ratio between the amount of glucose leaving the liver ( $Q$ ) and the amount passing through the liver ( $D$ ). Hence

$$\frac{\gamma_A}{\gamma_H} = \frac{Q}{D} \quad (1)$$

While  $D$  cannot be ascertained the value of  $Q$  is calculated from the estimated hepatic blood flow<sup>6</sup> and the hepatic venous glucose concentration, and further as

$$Q = D + a \quad (2)$$

where  $a$  is the amount of unlabeled glucose released from the liver, this amount can be derived as

$$a = Q \left( 1 - \frac{\gamma H}{\gamma I} \right) \quad (3)$$

This equation will be suitable for liver output calculations even in periods

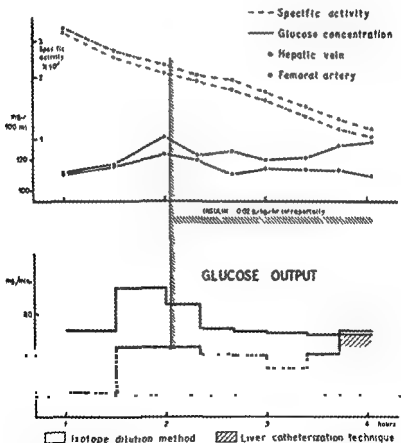


FIGURE 6. Effect of intraportal infused insulin (0.02 IU/kg/hr) into a normal dog upon the output of unlabeled glucose from the liver and the net amount of glucose leaving the splanchnic area. In the upper part of the figure are shown the arterial and hepatic venous plasma glucose concentration and specific activity.

where a steady state with respect to glucose metabolism is not maintained as variations in the glucose pool do not interfere

The output of unlabeled glucose from the liver before and during an intraportal infusion of 0.02 I U of insulin/kg/hour is shown in FIGURE 6 together with the net amount of plasma glucose leaving the splanchnic organs, calculated from the difference in glucose concentration of hepatic venous and arterial plasma multiplied by the estimated hepatic plasma flow. Although this dose of insulin does not have any apparent effect upon the arterial blood sugar concentration it could be anticipated that if insulin effects the glucose uptake of the liver cells this dose given intraportally might be followed by an alteration in the net amount of glucose leaving the liver, and also in its release of unlabeled glucose. This however was not the case as the values remained approximately constant for the whole infusion period although the hepatic cells presumably were exposed to concentrations of insulin that would have stimulated the glucose uptake of peripheral tissues.

Similar results were obtained with intraportal infusion of 0.04, 0.08 and 0.12 I U/kg/hour. The decrease in the arterial blood sugar indicates only an increased uptake of glucose in the peripheral tissues, as the output from the liver remained almost constant in this period. Thus the results obtained after intraportal infusion of relatively small doses of glucagon free insulin into cyclopropane anesthetized normal dogs indicate that the glucose output from the liver is not even partly inhibited by the influence of exogenous insulin.

### Discussion

The temporary stop in the decrease of the plasma glucose specific activity following tolbutamide injections into normal dogs and more so the longer lasting plateau observed in the pancreatectomized dogs are thought to be due to an inhibition of the output of unlabeled glucose from the liver. A similar inhibitory action of the sulfonylureas upon the hepatic release of glucose has been observed by Anderson *et al.*<sup>7</sup> Recant and Fischer,<sup>8</sup> and Ashmore *et al.*<sup>9</sup> Simultaneously a stimulatory effect upon the glucose uptake of the peripheral tissues could not be demonstrated, which is in contrast to the observations of Perre and Yalow<sup>10</sup> and of Ashmore *et al.*<sup>9</sup> as a single intravenous injection of insulin into normal dogs. The plasma glucose concentration was never associated with any decrease in the hepatic glucose output. Thus the stimulatory effect of insulin upon the glucose uptake in the periphery was responsible for the fall in the blood sugar. This was also evident from the increased rate at which the plasma glucose specific activity decreased after the insulin was administered, and confirms previous observations with C<sup>14</sup> glucose.<sup>9, 10</sup>

### Conclusions

As the hypoglycemic effect of the sulfonylureas is not associated with any such stimulation of the glucose uptake it must be concluded that their action is not likely to be exerted via an increase in the secretion of insulin from the pancreas or in the amount of insulin available in the body, but must be due

to its inhibitory effect upon the glucose output from the liver. However as Dulin and Johnson<sup>11</sup> and Sobel *et al*<sup>12</sup> have been able to demonstrate a hypoglycemic effect of sulfonylureas in the hepatectomized rat or dog the action upon the hepatic glucose output may not be the only mode of action of these compounds but it presumably plays a major role in the intact animal which is not able to mobilize more liver glycogen to counteract the hypoglycemia produced by the sulfonylureas.

### Summary

The influence of tolbutamide and insulin upon the hepatic glucose output and the glucose uptake in the peripheral tissues was studied in normal and pancreatectomized dogs and diabetic patients by applying the technique of single injection of C<sup>14</sup> glucose.

Tolbutamide produced a definite cessation in the constant dilution of the plasma glucose specific activity normally caused by the release of unlabeled glucose from the liver to replace the blood sugar taken up by the periphery as demonstrated by a plateau in the specific activity curve which was most pronounced in the pancreatectomized dogs and the diabetic patients. As no increase in the peripheral utilization of glucose was demonstrated in these preparations it was concluded that the hypoglycemic effect of tolbutamide is caused by an inhibition of the release of glucose from the liver.

By combining hepatic venous catheterization with the C<sup>14</sup> glucose technique a new method of calculating the hepatic glucose output was worked out. This was not affected by changes in the size of the glucose pool or the

100 µg/hour. In no instance was this followed by a decrease in the hepatic glucose output. The hypoglycemia was solely produced by a stimulation of the peripheral glucose utilization which is not observed after injections of tolbutamide.

It was therefore concluded that it is very unlikely that the sulfonylureas exert their hypoglycemic effect via an increased release of insulin from the pancreas.

### Acknowledgments

The authors express their indebtedness to K. Hallas Møller for many valuable suggestions and stimulating criticism and to A. Thordahl Christensen, The Royal Veterinary and Agricultural College, Copenhagen, for placing at their disposal the X-ray facilities of his department. The technical assistance of Kirsten Harder and of Lise Wollesen is also gratefully acknowledged.

### References

1. SCHAMBYE P. 1957. On the action of BZ III and D 860 in pancreatectomized dogs. *Diabetes* 11: 146.
2. SCHAMBYE P. & F. TARDING. 1957. Abstracts. *Acta Physiol Suppl* 42: 145.
3. SEARLE G. I. & I. L. CHALKOFF. 1952. Inhibitory action of hyperglycemia on

- delivery of glucose to the blood stream by liver of the normal dog *Am J Physiol* 170 456
- 4 CREUTZFELDT W L DEIERING & O WELTE 1957 Das B Zellsystem von normalen und hypophysectomierten Ratten sowie von Kaninchen unter D 860 und diabetogenen Hormonen *Deut med Wochenschr* 82 1564
  - 5 DUNN D F B FRIEDMAN A R MAASS G A REICHARD & S WEINHOUSE 1957 Effects of insulin on blood glucose entry and removal rates in normal dogs *J Biol Chem* 225 225
  - 6 BRADLEY S E F J INGELFINGER G P BRADLEY & J J CURRY 1945 The estimation of hepatic blood flow in man *J Clin Invest* 24 890
  - 7 ANDERSON G L A J PERFETTO R N MONACO & C M TERMINE 1957 Observations on the action of tolbutamide *Diabetes* 6 34
  - 8 REICANT L & G L FISCHER 1957 Studies on the mechanism of tolbutamide hypoglycemia in animal and human subjects *Ann N Y Acad Sci* 71(1) 62
  - 9 ASHMORE J G F CAHILL Jr A S EARLY & S ZOTTU 1958 Studies on the disposition of blood glucose *Diabetes* 7 1
  - 10 BERSON S A & R S YALOW 1957 Some remarks on the mechanism of action of the sulfonylureas *Diabetes* 6 274
  - 11 DILLIN W F & R L JOHNSTON 1957 Studies concerning the role of the liver in the hypoglycemic response of animals to tolbutamide *Ann N Y Acad Sci* 71(1) 177
  - 12 SOBEL G W J RODRIGUEZ INIGO J V MORTON & R LEVINE 1958 Studies on the action of the sulfonylureas in liverless dogs *Metabolism* 7 222

### Discussion of the Paper

SIDNEY WEINHOUSE (*The Institute for Cancer Research, Philadelphia, Pa*)

I appreciate this opportunity of describing some experiments published in the September-October 1958 issue of *Diabetes*. These were carried out by my colleagues Reichard, Jacobs and Friedmann. Like the studies already reported by James Craig and Leonard Madison, they suggest an important role for insulin, as well as for the synthetic hypoglycemic agents, in suppressing hepatic glucose output.

FIGURE 1 shows results of an experiment similar to those that have just been described by Schambye, indicating that in the normal human hepatic glucose output is suppressed by the intravenous injection of insulin in a dosage of 0.1 U/kg. With the administration of insulin there is an immediate drop in the blood sugar which coincides exactly in time with the

by a resumption in drop of specific activity and begins to level off. Approximate inflow and outflow rates can be calculated from the data presented; this is shown in the inset of the figure. The solid line represents the inflow rates; the dotted line represents the rate of disappearance, presumably due to peripheral utilization. When the insulin is administered in one dose, there is a profound lowering or disappearance of glucose inflow simultaneous with a large increase in glucose disappearance rate.

FIGURE 2 shows a similar experiment in which tolbutamide was administered. Experiments were not carried out with chlorpropamide, unfortunately, but we hope to do this. Here again it can be seen that, coinciding with the hypoglycemic action of the drug, there is a "plateauing" of specific activity of the blood glucose for a period of about 20 to 30 min, again indi-

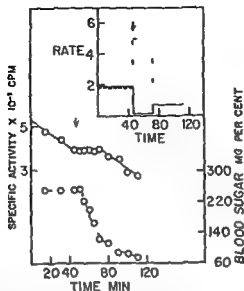


FIGURE 1 Blood sugar changes in a male subject weighing 60 kg and given 6 U of insulin (indicated by a row). Broken line represents blood glucose level; solid line specific activity. Inset shows approximate values with solid line representing rate of broken line removal.

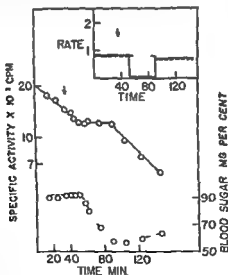


FIGURE 2 Blood sugar changes in a nondiabetic subject weighing 50 kg and given 12 gm tolbutamide intravenously (indicated by a row). Broken line represents blood glucose level; solid line specific activity. Inset shows approximate rates with solid line representing rate of broken line removal.



cating a suppression of glucose output. However, when the inflow and outflow rates are calculated, we find a significant difference from the experiments with insulin.

Although tolbutamide lowers the inflow of blood glucose, it does not have the stimulating effect on utilization observed when insulin was given. This apparent lack of stimulation of peripheral utilization represents a profound difference in the action of the two hypoglycemic agents.

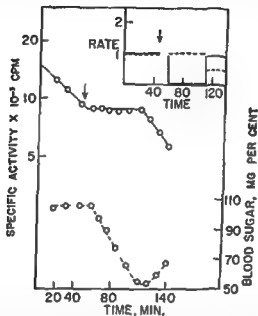


FIGURE 3 Effect on the time course of blood glucose concentration of 10 U of insulin given subcutaneously to a nondiabetic patient weighing 66 kg. Arrow indicates time of administration of insulin. Broken line represents blood glucose level, solid line specific activity. Inset shows approximate rates with solid line representing entry, broken line, removal.

It also occurred to us, as it did to other investigators, that endogenous insulin secreted slowly into the portal vein might have an effect different from that of intravenously injected insulin. To simulate the slow release of insulin that, presumably, takes place when tolbutamide is injected, we did some experiments in which we injected the same amount of insulin subcutaneously, so that there would be slow release into the blood stream.

inflow without a simultaneous increase in the utilization of glucose.

We interpret these results as indicating that the slow introduction of small doses of insulin into the blood stream causes a depression of the hepatic glucose output, but has little or no effect on the peripheral utilization of

glucose. We have also carried out experiments using slow intravenous infusion, and we have obtained identical results.

As others have indicated, these results do not exclude the possibility that tolbutamide or chlorpropamide have effects other than the stimulation of insulin release, but they do reconcile some of the previous results that were interpreted as indicating a different effect. More significantly, these results suggest to us that we should reconsider and re-evaluate the possibility of hepatic glucose output regulation in the problem of diabetes.

# COMPARISON OF SOME METABOLIC EFFECTS OF INSULIN, CHLORPROPAMIDE, AND OTHER HYPOGLYCEMIC SUBSTANCES\*

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Convincing experimental evidence suggests that carbutamide and tolbutamide stimulate the release of insulin from the pancreas<sup>1-16</sup>. However, this cannot be accepted as the sole explanation for the hypoglycemia because these drugs do not share other metabolic effects of exogenous insulin<sup>17-21</sup>. Two hypotheses may explain these differences in mode of action: (1) insulin secreted into the portal system as a result of normal pancreatic function or pancreatic stimulation acts differently from insulin injected peripherally, or (2) the drugs act not only on the pancreas but on the liver<sup>22-24</sup> or other tissues also. Experiments designed to study these hypotheses have given contradictory results. Two groups of workers compared the effects of insulin and tolbutamide on the rate of disappearance from the plasma of injected C<sup>14</sup> glucose. The first group<sup>25</sup> concluded that tolbutamide causes hypo-

insulin injected into the femoral vein. The other group of workers<sup>1</sup> confirmed the observation concerning tolbutamide and insulin injected intrave-

ly, the rate of disappearance of glucose injected subcutaneously was suppressed. Still another group<sup>26</sup> observed that tolbutamide increases hypoglycemia, increases peripheral vascular flow, and increases the rate of disappearance of glucose from the peripheral vein, thus

confirming the observation that the metabolic effects of insulin may vary, depending upon the route of administration. Unfortunately, these conclusions are based on the measurement of arteriovenous (A-V) differences without measurements of blood flow. Experiments performed in this labora-

tory have shown that the differences between the effects of insulin and tolbutamide on the rate of disappearance of glucose from the femoral vein of dogs are due to differences in the concentration of glucose in the portal blood and not to differences in the concentration of

absence of functioning pancreatic tissue. In the absence of functioning pancreatic tissue, the rate of disappearance of glucose from the femoral vein is not affected by tolbutamide.

mide and tolbutamide may act on the liver but only if small (permissive) quantities of endogenous or exogenous insulin are provided<sup>18, 27</sup> Essentially the same conclusion was reached recently by other investigators<sup>28</sup> This paper reports similar observations on the action of chlorpropamide

### Methods

rapid induction and the long lasting uniformly deep anesthesia with complete muscle relaxation that is essential to prevent fluctuations in blood pyruvate and lactate unrelated to the experiments Chlorpropamide\* was injected rapidly into the femoral vein in doses of 5 or 50 mg/kg or into the pancreatic artery in doses of 5 mg/ml/kg at the rate of 1 ml/min Control injections of saline into the femoral vein and of saline or glucose (0.5 gm in 20 cc) into the pancreatic artery had been performed previously<sup>27, 28</sup> Blood samples were taken from an exposed femoral vein for duplicate analyses Glucose was determined according to Nelson<sup>29</sup> pyruvate according to Friedemann and Haugen<sup>3</sup> lactate according to Barker and Summerson<sup>3</sup> and potassium with the Coleman flame photometer The carotid pressure of the dogs was used to check their general condition which remained satisfactory in all but two animals these were discarded The statistical significance of the results was calculated according to Fisher<sup>22</sup>

### Results and Discussion

The results are given in the form of curves representing average changes from preinjection values FIGURE 1 shows that the injections of saline or chlorpropamide in doses of 5 mg/kg into the pancreatic artery or into the femoral vein had no significant effect on peripheral blood glucose concentration In contrast the injection of glucose caused a significant ( $p < 0.01$ ) hypoglycemia The results suggest that contrary to the results obtained under comparable conditions with carbutamide and tolbutamide chlorpropamide does not stimulate insulin release FIGURE 2 shows that the injection of chlorpropamide in doses of 50 mg/kg into the femoral vein causes a prompt and significant decrease in the concentrations of blood glucose ( $p < 0.01$ ) and of lactate ( $p < 0.01$ ) and of potassium ( $p < 0.05$ ) d

not designed to determine whether the hypoglycemic action of chlorpropamide like that of carbutamide and tolbutamide requires the presence of small (permissive) quantities of insulin

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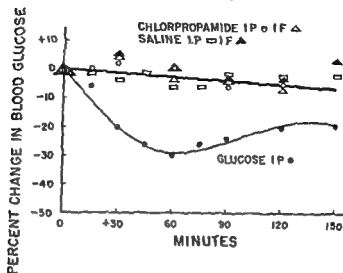


FIGURE 1 Effect of intrapancreatic (IP) or intrafemoral (IF) injections of chlorpropamide or saline (straight line) and of intrapancreatic injections of glucose (curve) on blood glucose

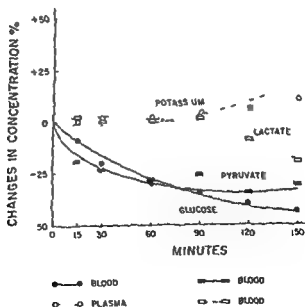


FIGURE 2 Effect of intrafemoral injections of chlorpropamide on blood glucose pyruvate lactate and plasma potassium

*Summary and Conclusions*

Chlorpropamide was injected into the pancreatic artery or into the femoral

arteries of blood glucose, pyruvate and lactate. The results are consistent with the hypothesis that the hypoglycemic effect of the drug is due, at least in part, to a decreased liver glucose production and offer no evidence for a pancreatic atrophic action of chlorpropamide.

*References*

1. FOÀ P P 1956 Derivatives of sulfonylureas with hypoglycemic action. *Chicago Med School Quart* 17: 145
2. LOUBATIÈRES A 1957 The mechanism of action of the hypoglycemic sulfonamides. *Diabetes* 6: 408
3. POZZA G G, GALASSINO & P P FOÀ 1956 Insulin secretion following carbutamide injections in normal dogs. *Proc Soc Exptl Biol Med* 111: 539
4. COLWELL A R JR, J A COLWELL & A R COLWELL SR 1956 Intra-pancreatic perfusion of the antidiabetic sulfonylureas. *Metabolism* 5: 749
5. CAPELLI V, G DOZIO & S VOLI 1956 Alcuni risultati di ricerche sul meccanismo d'azione dei sulfamidici ipoglicemizzanti. *Boll soc ital biol sper* 32: 916
6. HOLT C von L, von HOLT J, BRACHT B, KRÖNER & J KLINGAL 1957 Carbutamide and plasma insulin activity. *Science* 125: 735
7. GOETZ F C A, S GILBERTSEN & V JOSEPHSON 1956 Acute effects of Orinase on peripheral glucose utilization. *Metabolism* 5: 789
8. PINNELL R, J BRAY F, PRATT C, HAD JR & H CLERICUS 1956 Some observations on the mode of action of Orinase. *Metabolism* 5: 778
9. BANTIER P A, J R M FRANKSON, R DE MEUTTER, T C DEMANET & A CONARD 1957 Metabolic effects of carbutamide in selected diabetics. *Lancet* 1: 504
10. MILLER W L JR, J J KRAKE & M J LANDERBROOK 1957 Studies on the utilization of uniformly labeled C<sup>14</sup>-glucose by rats given tolbutamide. *J Pharmacol Exptl Therap* 119: 513
11. CASCIO G 1957 Azioni farmacologiche della N-(4 metil benzensulfonil) N-butilurea sul Colombo. I. Modificazioni della glicemia e del glicogeno epatico in funzione della dose. *Boll soc ital biol sper* 33: 1007
12. CREUTZFELDT W & H SÜTTERLI 1957 Vergleichende Untersuchungen über das Verhalten des Leber und Diaphragma Glykogens der Ratte unter Insulin und D 860. *Deut med Wochschr* 82: 1574
13. ASHMORE J G F, CAHILL JR A S, FARLE & S ZOTTI 1958 Studies on the disposition of blood glucose. A comparison of insulin and Orinase. *Diabetes* 7: 1
14. KELSEY FRY I & P H WRIGHT 1957 The action of hypoglycemic sulfonylureas on carbohydrate metabolism in the fasted rat. *Brit J Pharmacol Chemotherap* 12: 350
15. BRASSLER R & F L INGEL 1957 Some metabolic actions of Orinase. *Proc Soc Exptl Biol Med* 111: 738
16. HENNING A R, E S HANSEN, S S TAYLOR & J W CORRY 1957 Comparative effects of insulin and Orinase on blood levels of pyruvate and alpha ketoglutarate in normal subjects. *Metabolism* 6: 63
17. MOORHOUTSE J A M, R M KARS 1956 Physiologic actions of Orinase and their relationship to the types of diabetes in man. *Metabolism* 5: 847
18. STEIGERWALD H F, BORLE J, SCHOFFLING & F F PREIFFER 1957 Das Verhalten



## EFFECT OF CHLORPROPAMIDE ON INTERMEDIARY METABOLISM IN HUMAN SUBJECTS\*

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Development of the oral hypoglycemic agents has stimulated widespread investigation in an attempt to clarify their mode of action. As a consequence, the metabolic activities of insulin have been investigated more intensively. At the present time there are two general concepts about the action of the sulfonylureas. One concept is that the principal action of tolbutamide and carbutamide is to stimulate insulin release from the pancreas<sup>1,2</sup> the other is that these agents produce hypoglycemia by decreasing hepatic glucose output without increasing peripheral utilization<sup>3,4</sup>. Almost all metabolic studies reported in human and in experimental animals have been acute experiments, that is, the metabolic effects have been studied over a period of a few hours after the administration of the initial dose of the drug. It was felt that it would be worthwhile to investigate the metabolic effects of chronic administration of chlorpropamide in diabetic patients who were being maintained on it.

### *Methods and Materials*

Diabetic patients were selected from the general Diabetic Clinic at Jefferson Davis Hospital, a city county charity institution. Patients known to be free of metabolic disease and members of the professional staff served as normals. Where possible, studies were performed before as well as after chlorpropamide therapy was instituted. Each patient had received chlorpropamide at least one week before the "on treatment" study was performed. After an overnight fast the patients came to the metabolic laboratory and remained at rest in the laboratory until completion of the test. They took insulin and/or chlorpropamide in the dose of 500 to 1500 mg/day in their usual manner. Blood samples were obtained while the patient was fasting and at 1, 2 and 4 hours after administration. It is known that the composition of the diet will influence the blood levels of certain metabolites. It would be desirable to have all patients on a standard diet, but this is not possible because of the very limited resources available to this group of patients. Experience with these subjects has shown that the majority consume a rather constant diet that is low in protein and high in carbohydrate, fat, and calories,<sup>5</sup> for this reason no dietary instructions were issued and the patients were asked to remain on their usual diets.

In the majority of published studies glucose has been used as the test meal. In order to determine whether any other abnormalities would appear when a

\* The work reported in this paper was supported in part by a grant from Chas. Pfizer & Co. Inc. Brooklyn, N. Y.

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more normal dietary mixture was used the bulk of the data herein reported was obtained using a test breakfast containing 20 gm protein 65 gm carbohydrate and 20 gm fat. Additional studies utilizing glucose have also been performed for comparison. Analyses were performed for blood sugar,<sup>9</sup> inorganic phosphate,<sup>10</sup> pyruvate,<sup>11</sup> alpha ketoglutaric acid,<sup>11</sup> lactate,<sup>11</sup> total ketones,<sup>12</sup> and citrate.<sup>14</sup>

### Results

The blood sugar lowering effect of chlorpropamide is seen in FIGURE 1. It should be noted that after the test meal the normals did not develop any elevation in blood sugar which is probably due to delayed absorption. In contrast the diabetic developed significant elevations both before and after

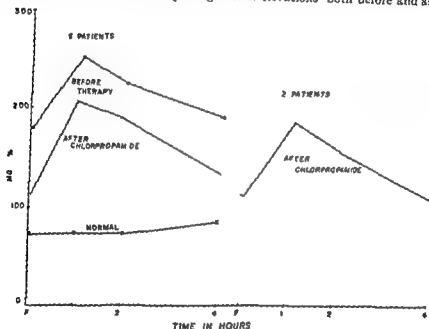


FIGURE 1 Blood sugar after test meal

therapy. However the after therapy curve is significantly lower than the before therapy curve although its shape is not altered greatly. In general these results are in agreement with those reported by other investigators utilizing tolbutamide and carbutamide in acute experiments.<sup>17</sup>

The studies on serum inorganic phosphate appear in FIGURE 2. In spite of the flat glucose tolerance curve the normals showed typical fall in phosphate following the meal and the values returned to normal by the fourth hour. In the insulin treated diabetics this response is still seen, but is less marked. In the chlorpropamide treated diabetics there is no change in serum phosphate level following the test meals. Statistical analysis indicates that there is no difference in the results obtained before and after therapy. These find

ings are in agreement with other reports<sup>3, 12</sup> on the effect of tolbutamide and carbutamide after acute administration

Pyruvate blood levels (FIGURE 3) normally rise after a meal with fall back to normal at 2 to 4 hours<sup>13, 16</sup> The same is true of the insulin treated diabetic The untreated diabetic also has essentially the same response The chlorpropamide treated diabetic however has an elevated fasting level of pyruvate that fell after ingestion of the meal This elevation is of borderline significance and appears in all of the experiments This fasting elevation of pyruvate in chronically treated patients has not been reported previously The data of Hennes *et al*<sup>11</sup> are not comparable, as these authors subjects were

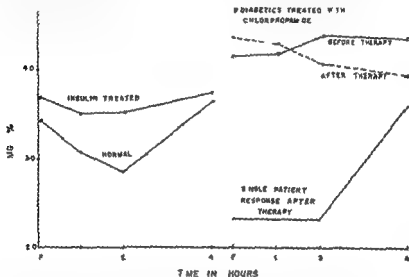


FIGURE 2 Inorganic phosphate

not receiving sulfonylureas on a chronic basis. The elevation in pyruvate if substantiated would indicate either increased production probably from gluconeogenesis or decreased utilization. As there has been no evidence that sulfonylureas increase gluconeogenesis it is probable that the elevated fasting pyruvate level is due to decreased utilization of pyruvate.

Under the conditions of these studies pyruvate, lactate, citrate, and alpha-ketoglutarate will vary considerably, and alterations would require rather considerable magnitude to be significant. The results on lactate appear in TABLE 1 and show that there is no significant alteration although there is considerable variability. The relatively high fasting values are probably due to recent activity of the patient in coming to the hospital rather than any drug induced effect.<sup>17</sup> In TABLE 2 it is seen that after the test meal the citric acid level rises at the first hour and then falls to normal. Other investigators have shown that the response of serum citrate in normal persons

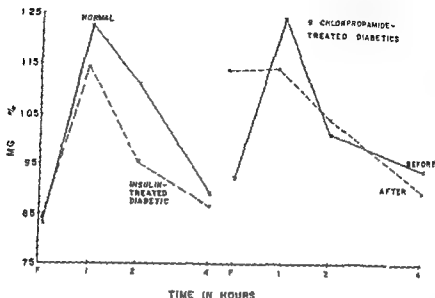


FIGURE 3. Pyruvate

after glucose feeding is a fall with a return to normal in 4 to 6 hours<sup>11, 19</sup> However, in diabetics there is often a rise after the meal. Glucose was fed to 6 normal individuals, and the serum citric acid levels fell as described in the literature<sup>18</sup> It appears that the rise in citrate in normals after the test meal is related to the diet. In diabetics, before treatment, the postprandial rise in citrate is rather large and prolonged, and this is not altered after treatment with either insulin or chlorpropamide. The studies on alpha ketoglutarate (TABLE 3) reveal no striking changes. The apparent elevated fasting levels after chlorpropamide therapy in the group that was studied before and after therapy would appear to approach significance, but the results obtained in the group studied only after chlorpropamide therapy do not support this

TABLE 1  
LACTATE  
(Time in hours)

	F	1	2	4	No pts
	Mean $\pm$ SF mg %				
Normal	11.1 $\pm$ 1.2	9.3 $\pm$ 0.62	9.3 $\pm$ 0.68	9.5 $\pm$ 1.13	6
Insulin	11.8 $\pm$ 1.3	12.9 $\pm$ 1.7	11.3 $\pm$ 1.5	11.8 $\pm$ 1.9	8
Chlorpropamide					
Before	10.9 $\pm$ 1.1	12.04 $\pm$ 1.3	12.5 $\pm$ 1.9	10.6 $\pm$ 1.4	12
After	9.9 $\pm$ 0.53	10.4 $\pm$ 0.66	10.8 $\pm$ 0.91	10.0 $\pm$ 0.75	15

TABLE 2  
CITRIC ACID  
(Time in hours)

	F	1	2	4	No pts
	Mean $\pm$ S.E. mg %				
Normal	2.1 $\pm$ 0.28	2.2 $\pm$ 0.16	2.01 $\pm$ 0.24	1.82 $\pm$ 0.13	6
Insulin	2.1 $\pm$ 0.13	2.8 $\pm$ 0.19	2.9 $\pm$ 0.22	2.6 $\pm$ 0.23	8
Chlorpropamide					
Before	2.6 $\pm$ 0.09	3.0 $\pm$ 0.25	3.2 $\pm$ 0.15	2.6 $\pm$ 0.10	9
After	2.3 $\pm$ 0.12	2.9 $\pm$ 0.24	2.8 $\pm$ 0.14	2.5 $\pm$ 0.23	9

TABLE 3  
ALPHA KETOGLUTARIC ACID  
(Time in hours)

	F	1	2	4	No pts
	Mean $\pm$ S.E. mg %				
Normal	0.45 $\pm$ 0.09	0.52 $\pm$ 0.18	0.35 $\pm$ 0.15	0.36 $\pm$ 0.14	6
Insulin	0.47 $\pm$ 0.10	0.69 $\pm$ 0.12			13
Chlorpropamide					
Before	0.41 $\pm$ 0.06	0.51 $\pm$ 0.07	0.40 $\pm$ 0.05	0.36 $\pm$ 0.07	9
After	0.54 $\pm$ 0.13	0.48 $\pm$ 0.05	0.46 $\pm$ 0.05	0.40 $\pm$ 0.05	9
After unpaired	0.44 $\pm$ 0.07	0.45 $\pm$ 0.05	0.35 $\pm$ 0.06	0.33 $\pm$ 0.05	12

TABLE 4  
KETONES  
(Time in hours)

	F	1	2	4	No pts
	Mean $\pm$ S.E. mg %				
Normal	1.95 $\pm$ 0.19	1.75 $\pm$ 0.48	2.17 $\pm$ 0.5	2.9 $\pm$ 0.93	6
Insulin	3.15 $\pm$ 0.56	2.24 $\pm$ 0.49	2.75 $\pm$ 0.66	2.8 $\pm$ 0.59	8
Chlorpropamide					
Before	4.62 $\pm$ 1.04	3.04 $\pm$ 0.61	2.83 $\pm$ 0.50	3.13 $\pm$ 0.68	12
After	2.95 $\pm$ 0.77	2.20 $\pm$ 0.31	2.08 $\pm$ 0.26	2.31 $\pm$ 0.30	12

The blood ketone levels appear in TABLE 4. It may be seen that there is increased ketone production in the untreated diabetic. This is returned to a more normal level by the administration of insulin or chlorpropamide. The insulin treated diabetics herein reported were in only fair control, and the chlorpropamide treated patients had been on this drug for approximately 1 to 2 weeks. With better control of the disturbed carbohydrate metabolic defect in diabetics the production of ketones is further diminished. For example, a study of 6 patients who were in excellent control after several months of chlorpropamide therapy showed a mean fasting blood ketone level of  $0.89 \pm 0.18$  (S.E.). This does indicate that the chlorpropamide has the ability to increase the utilization of carbohydrate to the point of reducing ketone production to low normal.

### Conclusions

The study of human intermediary metabolism in normal and diabetic patients before and after administration of insulin and chlorpropamide indicate that there are some differences between the metabolic action of insulin and chlorpropamide. The configuration of the glucose curve and the failure of the phosphate response indicate that chlorpropamide on chronic administration has essentially the same action as that of tolbutamide and carbutamide and that this action differs from that of insulin. In addition there is an elevation of fasting pyruvate level after the administration of chlorpropamide. The chronic administration of chlorpropamide effectively suppresses overproduction of ketones. No significant alterations were seen in citrate lactate and alpha ketoglutaric blood levels, however small differences would not appear under the conditions of these experiments.

### References

1. MADISON, L. L., B. COMBES, R. UNGER & N. KAFAN. 1959. The relationship between the mechanism of action of insulin and its effect on the portal circulation. *Ann. N. Y. Acad. Sci.* 12: 1-10.
2. GOETZ, I. C. & S. GILBERTSON. 1958. The effect of chlorpropamide on the metabolism of glucose in the rat. *J. Biol. Chem.* 233: 1-10.
3. R. I. G. W. THORN. 1956. Studies on Metabolism. 1: 757.
4. PURNELL, R. A. & R. M. KARK. 1956. Some observations on the mode of action of Orinase. *Metabolism* 5: 778.
5. MOORHOUSE, J. A. & R. M. KARK. 1956. Physiologic actions of Orinase and their relationship to the types of diabetes in man. *Metabolism* 5: 847.
6. ASHMORE, J. G. F., C. HILL, A. S. EARLY & S. ZETTL. 1958. Studies on the disposition of insulin and Orinase. *Diabetes* 7: 1.
7. CRAIG, J. S. & J. MOLLATH, H. WOODWARD & M. MILLER. 1958. Direct effects of tolbutamide and insulin on peripheral glucose uptake. *Diabetes* 7: 267.
8. DOBSON, H. I., H. S. LIPSCOMB, J. A. GREENE & H. T. ENGELHARDT. 1958. Socio-economic status and diabetes mellitus. *J. Chronic Diseases* 7: 413.
9. NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* 153: 375.
10. FISKE, C. H. & Y. SUBBAROW. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375.
11. CAVALLINI, D. & V. FRONTALI. 1951. Quantitative determination of keto-acids by paper partition chromatography. *Biochim. et Biophys. Acta* 12: 439.

- 12 BARKER S ■ & W H SUMMERSON 1941 The colorimetric determination of lactic acid in biological material *J Biol Chem* **138** 535
- 13 GREENBERG L A & D LESTER 1944 A macromethod for the determination of acetone and ketone bodies *J Biol Chem* **154** 177
- 14 NATELSON S J B PINCUS & J K LUGOVAY 1948 Microestimation of citric acid *J Biol Chem* **178** 745
- 15 HENNEMAN D H & J P BLUMER 1957 The pattern of intermediary carbohydrate metabolism in Cushing's syndrome *Am J Med* **23** 34
- 16 HENNES A R ■ I WAJCHENBERG S S FAJANS & J W CONN 1957 Comparative effect of insulin and Glucagon on blood levels of pyruvate and alpha ketoglutarate in normal subjects *Metabolism* **6** 61
- 17 HILLS O W M H POWER & R M WILDER 1952 Diabetes mellitus and Cushing's syndrome concentrations of lactate and pyruvate in blood after ingestion of glucose *Diabetes* **1** 151
- 18 NATELSON S J B PINCUS & J K LUGOVAY 1948 Response of citric acid levels to oral administration of glucose I & II *J Clin Invest* **27** 446 450

# METABOLIC EFFECTS OF CONTINUED ADMINISTRATION OF SULFONYLUREA DERIVATIVES IN SELECTED DIABETIC SUBJECTS INTERRELATIONS WITH GLUCAGON\*

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Although the mode of action of sulfonylureas is not completely understood, certain well established facts have emerged from the accumulated clinical and experimental data. For example several laboratories have demonstrated that the hypoglycemic action of sulfonylurea compounds is associated with a reduced output of glucose by the liver<sup>1-3</sup>. The mechanisms by which the reduced glucose output is accomplished have not been clearly established despite an intensive study of the problem. However, a decreased conversion of galactose or fructose to glucose by the liver has been observed during the period of hypoglycemia induced by administration of sulfonylureas<sup>4,5</sup> and this has suggested the possibility that selective interference with hepatic gluconeogenesis might contribute significantly to the hypoglycemia. Little if any information is available on the effect of sulfonylureas on conversion of amino acids to glucose in the liver.

In the present studies an attempt has been made to determine whether the sulfonylureas might inhibit hepatic gluconeogenesis from protein and amino acids as well as hepatic glycogenolysis. To this end the effect of sulfonylureas on the metabolic responses to repeated daily intramuscular injections of glucagon has been studied. The ability of glucagon to increase the daily urinary nitrogen excretion as well as to raise the daily blood and urinary sugar levels with and without the simultaneous administration of carbutamide has been studied in two diabetic patients<sup>6</sup> who exhibited a striking hypoglycemic response to carbutamide. The effects of glucagon and carbutamide on the daily fasting levels of serum inorganic phosphorus on the daily urinary excretion of reducing corticosteroids and on the daily balances of phosphorus potassium and sodium have also been investigated.

The rationale for the glucagon carbutamide experiments was provided by earlier work in this laboratory. Metabolic studies with fed diabetic patients<sup>7,8</sup> and with fasting experimental rats<sup>9</sup> have shown that increased protein catabolism seems to be an integral part of the hyperglycemic glycogenolytic response to fairly large doses of glucagon. Furthermore, studies of the rat have suggested that the increased protein catabolism induced by

\* The work reported in this paper was supported by grants from Eli Lilly and Company Indianapolis Ind and Chas Pfizer & Co Inc Brooklyn, N. Y. and Research Grant A-611 from the National Institute of Arthritis and Metabolic Diseases Public Health Service Bethesda Md.

† The results of such a study in one of the patients have been published in part in a preliminary report.<sup>6</sup>

glucagon is probably due to an enhanced hepatic gluconeogenesis from amino acids.

The second part of this paper will deal with the question of a possible hypoglycemic action of sulfonylureas in the severe, unstable diabetic receiving suboptimal doses of exogenous insulin. There is well-documented evidence showing that, in long-term studies, carbutamide and tolbutamide can lower the levels of glucose in blood and urine in depancreatized dogs receiving suboptimal doses of exogenous insulin,<sup>11-14</sup> but not in severe, unstable diabetes<sup>15-17</sup> in humans similarly treated with suboptimal doses of exogenous insulin. With the introduction of more potent hypoglycemic agents such as chlorpropamide, it was considered worthwhile to reinvestigate this problem. The effects of tolbutamide and chlorpropamide on carbohydrate and protein metabolism have been determined for two patients with severe, unstable, long standing diabetes, who were presumed to be critically lacking in endogenous insulin.

## METHODS

### *Subjects*

Four patients with diabetes mellitus served as subjects. One of the patients (R. E.) had the stable type of diabetes, the remaining three had the unstable type. The illness was of very recent onset in one of these unstable cases (A. F.), in the other two cases (E. R. and E. D.) it was of many years' duration. The subjects were classified as unstable or stable according to a criterion described in a previous paper.<sup>18</sup> Throughout the period of study, which in each case extended several months, the patients lived quietly in the metabolism ward of Strong Memorial Hospital under routine but rigidly controlled conditions. Physical activity was usually restricted to being up and about in the ward as the patients wished.

### *Diets*

Each patient received a weighed diet constant in chemical composition, the identical menu being served every day. The methods and rationale for calculating protein, fat, carbohydrate, total calories, and distribution of carbohydrate among the meals have been reported elsewhere.<sup>19</sup>

### *Insulin*

Insulin was administered regularly to each patient as a single daily dose of either NPH or lente insulin every morning before breakfast. Initially, the patients were placed on approximately the same dose of insulin that they had been receiving before hospitalization. Subsequently, the dose was adjusted to achieve optimal regulation of blood and urinary sugar levels. In order to achieve more satisfactory regulation of the levels of sugar in blood and urine patients A. F., E. R., and E. D. received also a small dose of crystalline insulin each morning before breakfast. Suboptimal regulation of blood and urinary sugar levels was achieved by appropriate reduction of the total daily dose of insulin to the point where the levels of blood and urinary sugar became



significantly higher than those obtained with optimal doses of insulin, b without the development of ketosis

### *Analytical Procedures*

All patients were weighed daily every morning before breakfast, and fasting blood sugar and 24 hour urinary excretion of sugar, nitrogen, phosphorus, potassium, and sodium were determined daily for each. A sample daily menu for each patient was analyzed for nitrogen, phosphorus, potassium, and sodium. In each case the average daily fecal excretion of nitrogen, phosphorus, potassium, and sodium was calculated from a pooled 5-day collection of feces. Carmine was used to mark the beginning and end of the period of collection. Daily balances for nitrogen, phosphorus, sodium, and potassium were obtained by subtracting the observed daily 24 hour urinary values and calculated average daily fecal values from measured amounts of nitrogen, phosphorus, sodium, and potassium in a sample diet. Fasting plasma, inorganic phosphorus, and 24 hour urinary excretion of reducing corticosteroids were determined daily in patients R E and A F. In patient R E the fasting blood levels of carbutamide and capillary blood sugar were also measured daily.

Capillary samples of blood were drawn from the finger tip, usually in duplicate within 1 or 2 min of the venipuncture using pipettes calibrated to contain 0.2 ml. The purpose of this procedure was to estimate the concentration of glucose in arterial blood (capillary blood was assumed to equal arterial blood in glucose concentration). The fasting levels of total plasma amino acids and chlorpropamide were determined daily in patient E D.

### *Chemical Methods*

Sugar in blood and urine was determined by the Shaffer-Hartman copper iodometric method,<sup>20</sup> using Somogyi's new copper reagent.<sup>21</sup> Urines were analyzed for total nitrogen in duplicate by a modified micro-Kjeldahl method. Diets and stools were analyzed for total nitrogen in triplicate by the macro-Kjeldahl method using the "wet" technique.<sup>22</sup> Sodium and potassium in diets, stools, and urines were determined by means of a Baird flame photometer. Inorganic phosphorus in plasma, urine, diet, and stool was measured by the method of Fiske and Subbarow.<sup>23</sup> Total reducing corticosteroids in urine were determined as follows: the urine was hydrolyzed for 48 hours at 47° C with  $\beta$ -glucuronidase after adjustment to pH 4.5 and buffering. After the steroids had been extracted in chloroform a "neutral extract" of the latter<sup>24</sup> was prepared. Finally, the corticosteroids were estimated colorimetrically by a modification of the Mader and Buck method<sup>25</sup> using blue tetrazolium (BT).<sup>26</sup> Plasma amino acid nitrogen was measured by the method of Frame *et al.*<sup>26</sup> Serum carbutamide levels were measured by a modification of the method of Marshall and Bratton<sup>27</sup> and serum chlorpropamide levels were determined by D. Iezzoni of Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

### *Glucagon*

Patients R E and A F were allowed to reach nitrogen equilibrium before the control data were obtained. After suitable periods (of several days)

duration) each patient received either 1 or 2 mg of an aqueous solution of glucagon intramuscularly every 8 hours 24 hours per day for 6 days. One such course of glucagon was administered during the period of optimal regulation of blood and urinary sugar levels with exogenous insulin as described above and one course was given during optimal regulation of sugar levels with carbutamide. The glucagon used in these studies was an approximately 50 per cent pure amorphous preparation\*. It contained between 0.005 and 0.05 U of insulin per milliliter of solution and bore the Lot No 208 158B 214A.

### Sulfonylureas

All of the sulfonylureas used in these studies were given by mouth daily in divided doses. Patients K E and A F received 2.0 gm and 2.5 gm of carbutamide\* respectively per day. Patient E R received 1.5 to 2.5 gm of tolbutamide† per day, and patient E D was given 2 separate courses of chlorpropamide‡ the first at 1.5 gm per day and the second at 2.0 gm daily. At least 2 or 3 days were allowed to elapse after beginning or stopping treatment with the drug before the metabolic data were obtained.

### Results

#### *Effects of Carbutamide on the Responses of Various Metabolic Indices to Glucagon*

The pertinent metabolic data obtained in patients K E and A F are shown in FIGURES 1 and 2 and in TABLES 1 and 2 respectively. All data are expressed as mean daily values  $\pm$  2 standard errors about the mean with the exception of the  $\frac{3}{1}$  ratios in FIGURE 1. The latter values are expressed

as the mean daily values  $\pm$  1 standard error about the mean. This was done to avoid negative values about the mean which would have occurred for some periods because of the marked variability in the daily observed values and because of the small size of the sample.

**Blood and urinary sugar levels.** Before comparing the responses of the blood and urinary sugar levels to glucagon when the patients were or were not receiving carbutamide it may be well to point out that administration of carbutamide caused a striking fall in the levels of fasting blood sugar and in the excretion of glucose. This is evident from a comparison of the blood and urinary sugar levels in periods 1 and 5 with those of periods 6 and 8 in FIGURE 1 and of the levels in period 4 with those of periods 5, 7 and 8 in FIGURE 2. In patient K E the regulation of both the fasting blood sugar and the amount of sugar excreted in the urine was equal if not superior to that achieved by the administration of 36 U of insulin daily and in patient A F the regulation by carbutamide was as good as or better than the administration of 100 U of insulin daily without carbutamide. In both cases the daily ingestion of 2.0 to 2.5 gm of carbutamide resulted ultimately in fasting blood sugar levels close to or within the normal range and the excretion of less than

\* Supplied by Eli Lilly and Company Indianapolis Ind

† Supplied by The Upjohn Company Kalamazoo Mich

‡ Supplied by Chas Pfizer & Co Inc Brooklyn N Y



## Izzo Metabolic Effects

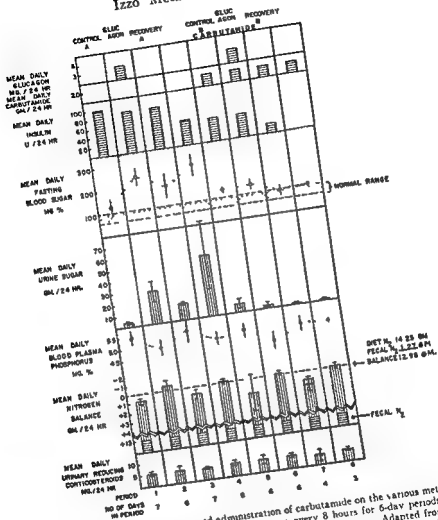


FIGURE 2 Effect of continued administration of carbutamide on the various metabolic responses to intramuscular injections of glucagon every 8 hours for 6-day periods. All data are expressed as mean daily values  $\pm 2$  SE about the mean. Adapted from Izzo and Roncone\* (by permission of *Diabetes The Journal of The American Diabetes Association*) Patient 3 I female 19 years old unstable diabetes short duration

fact that a smaller standard error was obtained with carbutamide than with insulin. Moreover the need for insulin in both patients is evident from the levels of hyperglycemia and the glycosuria that resulted when insulin was omitted (periods 1 and 5, FIGURE 1) or reduced to suboptimal levels (period 4, FIGURE 2).

Intramuscular injections of glucagon administered to both patients during

TABLE 1  
PHOSPHORUS, SODIUM AND POTASSIUM BALANCE DATA OF PATIENT R. F. FEMALE 64 YEARS OLD STABLE DIABETES OF LONG DURATION\*

No. of days	P x d:					
	1	2	3	4	5	6
Carbamide gm./24 hours	—	—	—	—	—	—
Chlucagon mg./24 hours	—	—	—	—	—	—
Inulin U./24 hours	—	—	—	—	—	—
Urinary inorganic phosphorus gm./24 hours	—	—	—	—	—	—
Urinary sodium mEq./24 hours	6285 ± 058	402 ± 120	600 ± 206	807 ± 124	806 ± 144	510 ± 216
Urinary potassium mEq./24 hours	637 ± 120	402 ± 120	548 ± 134	432 ± 68	610 ± 54	434 ± 92
Urinary potassium mEq./24 hours	744 ± 72	776 ± 80	872 ± 138	842 ± 36	876 ± 74	805 ± 70
* P further tests and results: All urinary values are expressed as the mean ± 2 S.D. about the mean.						
† T. Glucose in the daily balance subtracted the urinary values from the average daily intake; urinary values are less than the average daily intake and indicate negative balance.						
Average daily intake indicate negative balance						
Phosphorus gm						
Sample daily diet						
Average daily fecal excretion						
Average daily intake						
Potassium mEq						
Sample daily diet						
Average daily fecal excretion						
Average daily intake						
Sodium mEq						
Sample daily diet						
Average daily fecal excretion						
Average daily intake						

TABLE 2  
BALANCE DATA OF PATIENT A 1 FEMALE 19 YEARS OLD UNSTABLE DIABETES OF SHORT DURATION\*

No	Days	Ca Intake gm/24 hours	Glucose, mg/24 hours	Insulin U/24 hours	Urine nitrogen phosphorus gm/24 hours	Urine sodium mEq/24 hours	Urine potassium mEq/24 hours	P mod							
								1	2	3	4	5	6	7	8
100															
950 $\pm$ 136		340													
64.5 $\pm$ 21.4		986													
62.7 $\pm$ 3.0		29.1													
		104													
		28.0													
		5.5													
		174													
		39.0													
		5.6													
		131													
		27.0													
		8.4													
		114													
		5.8													
		4.1													
		945													
		114													
		1.006													
		64.2													
		75.5													
		0.074													
		176													
		19.8													
		19.8													
		9.7													
		118													

\* For further details see text and p. 65, fig. 2. All urinary values are expressed as the mean  $\pm$  2 S.D. about the mean.

† To obtain the daily balance subtract the urinary value from the average daily intake; values less than the average daily intake indicate positive balance and urinary values greater than the average daily intake indicate negative balance.

Sample daily diet: 1,377

Average daily fecal excretion: 540

Average daily intake: 837

the time of optimal regulation with exogenous insulin resulted in sharp increases in fasting blood sugar levels and 24 hour excretion of sugar (periods 2 and 3, FIGURE 1, and periods 1 and 2, FIGURE 2). The mean increase in daily fasting blood sugar above control levels was 101 mg per cent in patient R. E. and 119 mg per cent in patient A. F. The mean increases in daily urinary sugar levels were 7.31 gm and 24.47 gm, respectively. With discontinuation of glucagon the levels of blood and urinary sugar promptly moved toward the control levels (period 4, FIGURE 1 and period 3, FIGURE 2). Intramuscular administration of glucagon to patient R. E. during the time she was receiving carbutamide resulted in a mean increase in fasting blood sugar level, which was only slightly less than that caused by similar injections of glucagon during the time of optimal insulin regulation (periods 7 and 8, FIGURE 1). The mean rise in 24 hour excretion of sugar was 3.53 gm. It should be noted that the rise in the levels of fasting blood sugar induced by glucagon when patient R. E. was receiving either insulin or carbutamide was not associated with any increase in peripheral glucose utilization, as measured by the  $\frac{I}{A}$  ratio. In sharp contrast to the results obtained in patient

R. E., the administration of glucagon to patient A. F. at a time when she was receiving carbutamide with reduced doses of insulin produced no rise in levels of blood or urinary sugar (periods 5, 6, and 7, FIGURE 2).

glucose in the blood and urine glucagon also caused a significant rise in the excretion of nitrogen in the urine, with a reversal of nitrogen balance from positive to slightly negative (period 3, FIGURE 1 and period 2, FIGURE 2). The mean daily increase in the excretion of nitrogen during the period of glucagon administration was 2.39 gm higher than the control levels in patient 1 and 1.40 gm higher than the control levels in patient 2. With discontinuance of glucagon the excretion of nitrogen promptly decreased and the nitrogen balances again became positive (period 4, FIGURE 1 and period 3, FIGURE 2). It should be noted that patient R. E. was in positive nitrogen balance during periods 1 and 5, FIGURE 1 when insulin was omitted, even though the levels of hyperglycemia and glycosuria were similar to or even higher than the levels produced by glucagon and insulin. Glucagon also caused a greater excretion of nitrogen in patient A. F. than did reduction of insulin to suboptimal levels although the hyperglycemia and glycosuria were greater during the period of suboptimal insulin administration than during the period of administration of the optimal amount of insulin together with glucagon.

The administration of glucagon in the presence of carbutamide also resulted in a significant increase in the excretion of nitrogen in the urine in patients R. E. and A. F. The rises were roughly of the same order of magnitude as the rise in nitrogen excretion that occurred when the patients were given glucagon while receiving optimal doses of insulin. Nitrogen balances

reverted from positive to slightly negative in patient R E, and to equilibrium in the case of patient A F (periods 6 and 7, FIGURE 1, and periods 5 and 6 FIGURE 2). The increase in the mean daily nitrogen excretion induced by administration of glucagon was 1.29 gm in patient R E and 1.67 gm in patient A F. As in the glucagon-insulin studies, nitrogen excretion promptly decreased and the nitrogen balances again became positive when the injections of glucagon were stopped (period 8 FIGURE 1 and period 7 FIGURE 2). It is of interest to note that carbutamide did not diminish the nitrogen response to glucagon in patient A F even though it virtually abolished the hyperglycemic glycosuric response to glucagon. Nitrogen retention was greater in patient A F when she was receiving carbutamide and reduced doses of insulin than when carbutamide alone was given, although the regulation of blood and urinary sugar levels in both periods 7 and 8 FIGURE 2, was similar. However, because periods 7 and 8 were of short duration, little significance can be attached to these differences in nitrogen excretion.

*Plasma inorganic phosphorus.* Before discussing the effect of carbutamide on the response of the level of plasma inorganic phosphorus to the administration of glucagon, it is pertinent to point out the occurrence of an inverse relationship between the level of fasting blood sugar and the level of fasting plasma inorganic phosphorus in both patients R E and A I. This is shown in patient R E by the significant rise in mean daily level of plasma inorganic phosphorus from 4.11 mg per cent to 4.88 mg per cent as the

actively the phosphorus during the period of suboptimal insulin therapy (period 4 FIGURE 2) were 2.63 mg per cent and 4.98 mg per cent respectively. In fact, a close inspection of the mean daily levels of fasting blood sugar and plasma inorganic phosphorus for all periods in both FIGURES 1 and 2 suggest, with a few exceptions described below, that there is an inverse relationship between these two variables irrespective of the type of treatment. The glucose-phosphorus relationships just described are similar to those reported previously in diabetes.<sup>10-12</sup> They are of interest since they are the reverse of the well known short term relationship between glucose and phosphorus which has been observed in nondiabetics following the administration of glucose or insulin.

in levels of plasma inorganic phosphorus (periods 2 and 3 FIGURE 1, and periods 1 and 2 FIGURE 2). The mean decreases in levels of plasma inorganic phosphorus in patients R E and A I were 0.76 and 0.56 mg per cent, respectively. Discontinuation of glucagon resulted in a prompt return of plasma inorganic phosphorus to control levels (period 4, FIGURE 1, and



period 3, FIGURE 2) The administration of carbutamide did not alter the hypophosphatemic response to injections of glucagon in either patient (period 7, FIGURE 1, and period 6, FIGURE 2), even though, in one of these patients (A F), carbutamide virtually abolished the hyperglycemic response to glucagon administration The decreases during glucagon administration in the mean daily levels of serum inorganic phosphorus below the control levels in patients R E and A F were 1.02 mg and 0.63 mg per cent respectively As was the case with glucagon administration during period of optimal insulin therapy, discontinuation of glucagon resulted in a prompt return of serum inorganic phosphorus toward control levels It should be noted that in patient R E the fall in serum inorganic phosphorus induced by glucagon in periods 3 and 7 was not associated with any significant increase in peripheral glucose utilization as measured by changes in the

$$\frac{1}{1} \text{ ratio}$$

*Urinary reducing corticosteroids* The mean daily urinary excretion of reducing corticosteroids was within the normal range in both cases Glucagon administration produced a slight but significant rise in the mean daily excretion of urinary reducing corticosteroids in the case of patient R E when she was being treated with optimal doses of insulin (periods 2 and 3, FIGURE 1), but this did not occur when glucagon was again administered while the patient was receiving carbutamide instead of insulin (periods 6 and 7, FIGURE 1) Neither glucagon nor carbutamide had any effect on the level of excretion of urinary reducing corticosteroids in patient A F

*Phosphorus, potassium and sodium balances* Slight increases in the mean daily urinary excretion of phosphorus were observed when both patients R E and A F were given glucagon either while receiving optimal doses of insulin or carbutamide in periods 2, 3, 4, 6, 7, and 8 in Table 1, and in periods 1, 2, 3, 5, 6 and 7 in Table 2 The increases in inorganic phosphorus excretion induced by glucagon were highly significant ( $p < 0.01$ ) in the case of patient R E (only when carbutamide was given) but were not statistically significant ( $p > 0.05$ ) in the case of patient A F Although the daily balances for sodium and potassium varied markedly from day to day and period to period in both patients, no relationship could be established between the changes in balances of sodium and potassium and the administration of glucagon and carbutamide either singly or together

#### *Metabolic Effects of Sulfonlureas in Patients with Severe, Long-Standing, Unstable Diabetes of the Juvenile Type*

*Tolbutamide* The oral administration of 2.0 gm of tolbutamide daily to patient E R (FIGURE 3) for 12 days did not lower the levels of blood and urinary sugar (periods 1 and 2) In fact, the glycosuria tended to increase while the patient was receiving tolbutamide It should be pointed out that this patient was receiving only sufficient insulin daily to maintain the fasting blood sugar at slightly above the normal range, and the mean 24 hour excretion of glucose in the urine at approximately 25 gm Ketonuria was

taneous fall in levels of blood and urinary sugar (period 3) the nitrogen

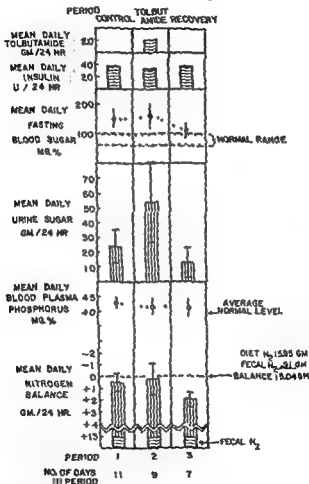


FIGURE 3 Effect of continuous administration of tolbutamide in a severe unstable diabetic maintained on suboptimal daily doses of insulin. All data are expressed as mean daily values  $\pm 2 S I$  about the mean. Patient F, male, 50 years old, unstable diabetes long duration.

balance became more positive even though the daily dose of insulin was

**Chlorpropamide** This compound was administered orally to patient E D during 2 different periods separated by an adequate recovery interval (FIGURE 4). On 24 U of insulin daily (20 U of lente insulin plus 4 U of crystalline insulin), the mean daily fasting blood sugar level during the 11 day control period was 247 mg per cent and the mean daily excretion of sugar in the urine was 50.2 gm (period 1). With the administration of 1.0 to 1.5 gm of chlorpropamide per day in addition to the 24 U of insulin per day for 16 days (period 2) the mean fasting daily blood sugar level was 188 mg per

TABLE 3

PHOSPHORUS SODIUM AND POTASSIUM BALANCE DATA OF PATIENT F R MALE  
50 YEARS OLD UNSTABLE DIABETES OF LONG DURATION\*

No of days	Period		
	1	2	3
	11	10	6
Tolbutamide gm 24 hours		2.0	
Insulin U 24 hours	36	34	36
Urine inorganic phosphorus gm 24 hours	796 ± 048	794 ± 090	756 ± 106
Urine sodium mEq 24 hours	80.4 ± 13.6	96.5 ± 16.4	76.6 ± 16.4
Urine potassium mEq 24 hours	89.4 ± 27.2	88.4 ± 33.8	80.4 ± 21.2

\* For further details see text and FIGURE 3. All urinary values are expressed as the mean  $\pm$  2 S.E. about the mean.

† To obtain the daily balance subtract the urinary value from the average daily intake. Urinary values less than the average daily intake indicate positive balance and urinary values greater than the average daily intake indicate negative balance.

	Phosphorus gm	Potassium mEq	Sodium mEq
Sample daily diet	1.017	98.52	102.62
Average daily fecal excretion	-1.054	- 3.43	- 1.78
Average daily intake	863	90.09	100.84

cent and the mean daily excretion of sugar was 26.7 gm. The decrease in level of fasting blood sugar was just significant at the 5 per cent level while the decrease in the amount of glucose excreted in 24 hours was significant at less than the 5 per cent level. With the discontinuation of chlorpropamide, both the mean daily fasting blood sugar and the mean 24 hour excretion of sugar returned toward control values (period 3). The mean daily fasting blood sugar level in period 3 was 231 mg per cent and the mean daily excretion of glucose 41.5 gm. After a 10-day recovery interval chlorpropamide was administered again (period 4). On this occasion the daily dose of chlorpropamide was increased to 2.0 gm per day while the daily dose of lente insulin was decreased to 16 U per day, with the 4 U of crystalline insulin omitted. There was no change in the 24 hour excretion of glucose

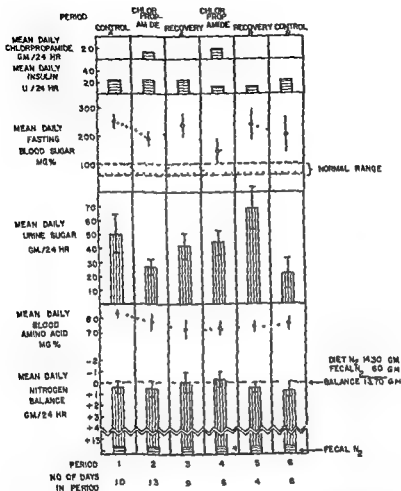


FIGURE 4 Effect of continued administration of chlorpropamide in a severe unstable diet = maintenance on suboptimal daily doses of insulin. All data are expressed as mean daily values  $\pm$  S.F. about the mean. Patient T.H. female, 31 years old, unstable diabetes long duration.

in the urine (43.9 gm in period 4 as compared to 41.5 gm in period 3) but the fasting blood sugar fell from a mean daily value of 234 mg per cent in period 3 to 142 mg per cent in period 4. The decrease of 92 mg in the mean daily fasting blood sugar level was significant beyond the 5 per cent level. When the chlorpropamide was discontinued but the daily dose of insulin maintained at 16 U of lente insulin per day the fasting blood sugar level moved toward but did not exceed the control levels, whereas the mean daily

tion as measured by changes in the  $\Delta I$  glucose difference. This suggests that the phosphorus was not entering into the glycolytic cycle of peripheral tissues. It is possible that, under the conditions described, the fall of inorganic phosphorus may represent entry of phosphorus in the glycogenic cycle in the liver, and that glucagon may be involved in the regulation of these processes. A different type of study would be needed to establish or disprove the validity of this hypothesis.

The failure of tolbutamide to lower blood or urinary sugar levels in patient E R is in agreement with the results of other investigators who have been unable to demonstrate a hypoglycemic action of tolbutamide or carbutamide in severe, unstable diabetics.<sup>16, 17</sup> For this reason the lowering of blood and urinary sugar levels in patient E D, which appeared to be associated with the administration of chlorpropamide, is of interest. The results suggest the possibility that some types of sulfonylurea compounds may have a hypo-

glycemic response to chlorpropamide on the basis of stimulation of the insulinogenic function of the pancreas. It is reasonable to suspect that a different type of mechanism may be involved. Chlorpropamide did not affect the daily nitrogen balances in this patient. However we do not consider this strong evidence against the possibility of an 'insulinlike' action of the sulfonylureas because the patient remained in nitrogen equilibrium while she was being suboptimally regulated with insulin without chlorpropamide.

The findings that have been discussed are not all readily explainable on the basis of a single locus of action of sulfonylureas. As pointed out, the probable hypoglycemic action of chlorpropamide in patient E D, who was presumed to be critically deficient in endogenous insulin, and the inhibition of the hyperglycemic response to glucagon by carbutamide in patient A F are difficult to explain on the basis of stimulation of the beta cells by the sulfonylureas to produce more insulin. Nevertheless, the results of this study are not inconsistent with the hypothesis that the main action of sulfonylurea compounds is in the pancreatic islets. This does not necessarily mean, however, that the sulfonylureas stimulate the beta cells to release or secrete more insulin as some investigators have suggested.<sup>21, 22</sup> It seems possible, for example, that the sulfonylureas might act by decreasing the ratio of glucagon to insulin through an inhibiting action on glucagon producing cells. However, a hepatic effect of glucagon cannot yet be excluded on the basis of the work reported above.

According to available evidence, the hypoglycemic action of sulfonylureas

of glucose

increasing the permeability of peripheral tissues for sugar, the findings described have been interpreted by some investigators as evidence that the hypoglycemic action of sulfonylureas is not insulinlike and, therefore, not the result of stimulation of beta cells by sulfonylureas.<sup>23, 27</sup> This conclusion

seems reasonable to us, but some investigators<sup>8</sup> hold that the sulfonylureas stimulate the beta cells to release or secrete small quantities of insulin directly into the portal venous system. This small amount of released endogenous

peripheral glucose uptake as measured by available techniques. It should be pointed out, however, that a direct action of insulin on the liver has not yet been unequivocally established.<sup>22</sup> It appears to us that a more plausible

Glucagon is known to have a well defined action on the liver, but as yet no action has been established for peripheral tissues.

One of the chief arguments raised to refute the possible action of sulfonyl urea compounds on glucagon production is that the compounds have no hypoglycemic effect in alloxan diabetic animals<sup>24</sup> in which presumably, the alpha cells are intact. This may not be a sufficient basis for excluding the hypothesis of an alteration in the glucagon:insulin ratio as a mechanism of action of sulfonylureas; however, it may well be that some insulin must be present in order that the effects of a diminution in glucagon may be observed. This idea seems rational because, in the absence of insulin, the utilization of glucose by the periphery is too small to cause a lowering of blood sugar sufficient to expose the failure of glucagon to act.

#### SUMMARY AND CONCLUSIONS

The metabolic effects of prolonged administration of carbutamide, tolbutamide, and chlorpropamide have been investigated in four selected diabetic subjects under controlled conditions in the metabolism ward of the Strong Memorial Hospital.

Except for an effect on blood and urinary sugar levels, none of the compounds that were studied appeared to have any direct or consistent effect on the following metabolic parameters: (1) daily balances of phosphorus, potassium, and sodium; (2) daily balances of nitrogen; (3) daily levels of fasting plasma  $\alpha$  amino acid nitrogen; and (4) daily urinary excretion of corticosteroids.

The effects of sulfonylureas on hepatic gluconeogenesis and glycogenolysis were investigated in two diabetic subjects who showed a striking hypoglycemic response to carbutamide. This was done by studying the effect of prolonged administration of carbutamide on the ability of repeated daily intramuscular injections of glucagon to increase the urinary excretion of nitrogen as well as to increase the levels of glucose in blood and urine. The influence of carbutamide on the ability of glucagon to depress the level of serum inorganic phosphorus was also investigated. Previous studies from this laboratory had demonstrated that the increased protein catabolism that is an integral part of the hyperglycemic glycogenolytic action of glucagon is related to an enhanced hepatic gluconeogenesis from protein.

Carbutamide markedly diminished the hyperglycemic and glycosuric response to glucagon in one patient but had no effect on this response in the other patient even though the drug had produced markedly decreased levels of blood and urinary sugar in both patients. However, in neither patient was there any influence of carbutamide on the increased protein catabolism or the depression of serum phosphorus caused by the administration of glucagon. These results suggest that the well established action of sulfonylureas in reducing hepatic glucose output is not dependent upon either an inhibition of the hyperglycemic glycogenolytic action of glucagon or a selective interference with hepatic gluconeogenesis from protein.

The present studies confirm the previous reports from this laboratory of the occurrence of an inverse relationship between the levels of fasting blood

The possible role of

administration of tolbutamide and chlorpropamide to reduce the levels of blood and urinary glucose in two cases of severe unstable diabetes where there was presumed to be a critical deficiency of insulin. Both patients were maintained on suboptimal doses of insulin. One patient was given tolbutamide and the other chlorpropamide. Tolbutamide was ineffective in reducing the levels of glucose in blood or urine. Chlorpropamide administration was associated with significant lowering of levels of blood and urinary sugar. The findings suggest that the hypoglycemic action of sulfonylureas may not be entirely dependent upon remaining islet cell reserve and that the capacity may vary with different compounds.

The metabolic effects of sulfonylureas that have been described are inconsistent with the hypothesis of a single mechanism of action such as a stimulation of the beta cells to secrete or release insulin. However the results do not preclude the hypothesis that the central locus of action of sulfonylureas is pancreatic islet tissue. It is possible that the action of sulfonylureas in reducing hepatic glucose output apparently without increasing peripheral glucose utilization is due to a reduction in the ratio of secreted glucagon to secreted insulin rather than to stimulation of the beta cells. This possibility is analyzed.

#### ACKNOWLEDGMENT

I express my appreciation of the assistance of Angela M. Roncone in the preparation of this paper.

#### REFERENCES

1. ANDERSON, G. F., A. J. PERFETTO, C. M. TERMINE & R. R. MONACO. 1956. Hypoglycemic action of Orinase: effect on output of glucose by liver. *Proc. Soc. Exptl. Biol. Med.* **92**: 340.
2. REICANT, L. & G. L. FISCHER. Studies on the mechanism of tolbutamide hypoglycemia in an animal and human subjects. 1957. *Ann. N. Y. Acad. Sci.* **71**(1): 62.
3. VAUGHAN, M. The effect of tolbutamide on glucose production by the liver in man. 1957. *Ann. N. Y. Acad. Sci.* **71**(1): 112.
4. HUMORE, J. G. F. CAMILL, JR., A. S. FARLE & S. ZOTTU. 1958. Studies on the disposition of blood glucose. A comparison of insulin and Orinase. *Diabetes* **7**: 1.

- 5 JACOBS G G REICHARD L H GOODMAN JR B FRIEDMANN & S WEINHOUSE 1958 Action of insulin and tolbutamide on blood glucose entry and removal Diabetes 7 358
  - 6 RENOLD A E & I WINEGRAD E R FROESCH & G W THORN 1956 Studies on the site of action of the arylsulfonylureas in man Metabolism 5 757
  - 7 MOORHOUSE J A & R M KARR 1956 Physiological actions of Orinase and their relationship to the types of diabetes in man Metabolism 5 847
  - 8 IZZO J L A RONCONE & M A PALIANI 1957 Effect of glucagon in diabetes Federation Proc 16(859) 200
  - 9 IZZO J L & A RONCONE 1957 Metabolic effects of carbutamide in diabetes and interrelations with glucagon A preliminary report Diabetes 6 45
  - 10 IZZO J L & S R GLASSER 1958 Influence of glucagon on protein metabolism of fasting rats Federation Proc 17(306) VII
  - 11 ROOF M A 1955 Animal experiments with BZ 55 1st Lilly Conf Carbutamide Indianapolis Ind
  - 12 SIREK A & O V SIREK 1956 The action of BZ 55 in dogs I Observations on depancreatized and Houssay dogs Can Med Assoc J 74 960
  - 13 CAMPBELL J 1956 The action of BZ 55 in dogs II Observations on depancreatized and metaphophyseal diabetic dogs Can Med Assoc J VII 962
  - 14 RICKETTS H T H L WILDBERGER & H SCHMID 1957 Long term studies of the sulfonylureas in totally depancreatized dogs Ann N Y Acad Sci 71(1) 170
  - 15 KUHLE W J JR 1956 Metabolic studies with arylsulfonylureas Metabolism 5 951
  - 16 CHUTE A L & H W BAIN 1956 Experiences with oral sulfonamide (BZ 55) in the management of juvenile diabetes Can Med Assoc J 74 994
  - 17 WILDBERGER H L & H T RICKETTS 1956 Effects of sulfonylurea drugs in hospitalized diabetic patients J Am Med Assoc 162 1045
  - 18 IZZO J L S L CRUMP & W KUNZ 1950 A clinical comparison of modified insulins J Clin Invest 29 1514
  - 19 IZZO J L I HODGES & A C SOKAL 1955 The
- ED 5-
- 21 SOMOGYI M 1945 A new reagent for the determination of sugars J Biol Chem 160 61
  - 22 REIFENSTEIN E C T ALBRIGHT & S L WELLS 1945 The accumulation interpretation and presentation of data pertaining to metabolic balances notably those of calcium protein and nitrogen J Clin Endocrinol 5 367
  - 23 FISKE C H & S SUBBAROW 1925 The colorimetric determination of phosphorus J Biol Chem 68 375
  - 24 IZZO J L F H KEUTMANN & R B BURTON 1957 Special considerations in the use of blue tetrazolium for the measurement of alpha ketols in mixtures with other steroids J Clin Endocrinol Metabolism 17 889
  - 25 MADER W J & R R BLACK 1952 Colorimetric determination of cortisone and related steroids Analytic Chem 24 666
  - 26 FRAME E G J A RUSSELL & A F WILHELM 1943 The colorimetric estimation of amino nitrogen in blood J Biol Chem 149 255
  - 27 BRATTON A C & E L MARSHALL 1939 A new coupling component for sulfanilamide determination J Biol Chem 128 537
  - 28 IZZO J L A M RONCONE & A GILERS 1956 Interrelationships of glucose and inorganic phosphorus in blood and urine of patients with diabetes mellitus Proc Soc Exptl Biol Med 91 373
  - 29 FAJANS S S L H LOUIS H S SELTZER R D JOHNSON R D GITTLER A R HENNES B L WACHENBERG I R ACKERMAN & J W CONN 1956 Metabolic effects of arylsulfonylurea compounds in normal men and diabetic subjects Metabolism 5 820



- 30 BERTNEY J E W SUTHERLAND & M H MAMMAN 1956 Observations on the action of certain sulfonylurea derivatives *Metabolism* 5 768
- 31 VOLK M W S WEISENFELD S R LAZARUS & M G GOLDBER 1956 Mechanisms of action of the hypoglycemia producing sulfonylurea derivatives *Metabolism* 5 894
- 32 FRITZ I B J A MORTON M WEINSTEIN & R LEVINE 1956 Studies on the mechanism of action of the sulfonylureas *Metabolism* 5 744
- 33 COLWELL A R JR J A COLWELL & A R COLWELL SR 1956 Intrapancreatic perfusion of the antidiabetic sulfonylureas *Metabolism* 5 749
- 34 DELIN W C & E L JOHNSTON 1957 Studies concerning the role of the liver in the hypoglycemic response of animals to tolbutamide *Ann N Y Acad Sci* 71(1) 177
- 35 RENOLD A G, D B MARTIN B R BOSHELL & G W THORV 1956 Studies on the site of action of the arylsulfonylureas in man II *Ann N Y Acad Sci* 71(1) 71
- 36 CRAIG J W A J MOLLAN H WOODWARD JR & M MILLER 1958 Direct effects of tolbutamide and insulin on peripheral glucose uptake *Diabetes* 7 267
- 37 ASHMORE J G F CARILL JR A S FARLE & S ZOTTI 1958 Studies on the disposition of blood glucose A comparison of insulin and Orinase *Diabetes* 7 1
- 38 MAHLER R W C SKOEMAKER & D M PUGH 1958 The effect of insulin on hepatic glucose production in normal dogs *Abstr No 66 40th Meeting Endocrine Soc*

# METABOLIC STUDIES OF CHLORPROPAMIDE IN NORMAL MEN AND IN DIABETIC SUBJECTS\*

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We have conducted short term and prolonged studies in normal men and in diabetic subjects to compare the physiological properties of chlorpropamide and tolbutamide. The primary objectives of the investigation were first, to correlate the hypoglycemic effect with the serum sulfonylurea level for each compound and, second, to compare the rates of disappearance of the two drugs from the blood. In addition, we performed metabolic balance studies in two normal men during the administration of chlorpropamide.

## Methods

**Serum sulfonylurea determinations** Serum concentrations of both chlorpropamide and tolbutamide were determined by the same analytical method. This method, developed by Toolan and Wagner for chlorpropamide and described elsewhere in this monograph, was found to serve equally well for the determination of tolbutamide.

The performance of the method in this laboratory is summarized in TABLE 1.

TABLE 1  
PERFORMANCE OF THE ANALYTIC METHOD IN THE DETERMINATION OF SERUM CHLORPROPAMIDE AND TOLBUTAMIDE CONCENTRATIONS

<i>Recovery of the sulfonylureas from serum</i>	
Chlorpropamide	97.0 ± 1.2%
Tolbutamide	99.0 ± 0.8%
<i>Features of the method</i>	
<i>Reproducibility (standards)</i>	
±0.25 mg./100 ml. sulfonylurea	
<i>Precision (intra-assay serum replicates)</i>	
±0.23 mg./100 ml. sulfonylurea	
<i>Accuracy (inter-assay serum duplicate)</i>	
±0.23 mg./100 ml. sulfonylurea	

0.5 mg./100 ml. scattered among 13 sets.

The recovery of both sulfonylureas added to serum was essentially complete.

The method which showed excellent reproducibility, allowed a precision of approximately 0.1 mg per cent and an accuracy of approximately 0.25 mg per cent in serum measurements of sulfonylurea.

Additional data relevant to the application of the serum sulfonylurea method are provided in TABLE 2. Chlorpropamide and tolbutamide, as well as tolbutamide and the tolbutamide metabolite, are interrelated in terms of their relative absorbance intensity in the method. A low serum "blank" value was found.

TABLE 2  
DATA RELEVANT TO THE APPLICATION OF THE SERUM SULFONYLUREA ANALYTICAL METHOD

Comparative quantitation data

	Relative absorbancy (on a weight basis)	
	100 $\mu$	—
Chlorpropamide	100	—
Tolbutamide	83.8	100.0
Tolbutamide metabolite	—	38.5
Serum blank values (35 different serum samples from 5 normal, 2 diabetic and 2 other subjects run in 22 sets) 0.22 $\pm$ 0.54 mg/100 ml (as chlorpropamide)		
Method not corrupted by		
(1) Products from hemolysis		
(2) Existence of the tolbutamide metabolite		

Mean  $\pm$  standard deviation given for serum blank values

The method is not corrupted by the products from hemolysis. Aliquots of 2 different blood samples devoid of sulfonylurea were briskly beaten with wooden applicators while the blood was clotting. The resultant deeply pigmented sera when compared in analysis with nonhemolyzed "duplicates," were found to have increased in apparent sulfonylurea only by about 0.4 mg per cent. The degree of hemolysis produced deliberately in this evaluation was approximately 10 times greater than any experienced throughout the investigation being described. Mention is made of this observation concerning hemolysis, since plasma and serum tolbutamide methods in current use have a demonstrated<sup>1</sup> or likely<sup>2,4</sup> sensitivity to corruption by the products from hemolysis.

As an important consideration, the sulfonylurea method used in our studies is not corrupted by the existence of the tolbutamide metabolite<sup>5</sup> (1-butyl-3-*p*-carboxyphenylsulfonylurea). The metabolic half life of the tolbutamide metabolite was determined by techniques similar to those described below for tolbutamide. It was found to be approximately 9 times (8.6) shorter than the metabolic half life of tolbutamide. The metabolite thus disappears from blood approximately 9 times faster than tolbutamide and exhibits absorbancy approximately 2½ times less than tolbutamide in this method (TABLE 2). It is unlikely therefore, that measured serum sulfonylurea levels, after tolbutamide administration, could be more than about 4 per cent in error because of the presence of the metabolite.

*Other determinations.* Fasting blood glucose, serum sodium, potassium, and chloride and urinary nitrogen, creatinine, sodium, potassium, chloride,

17-hydroxycorticoids, and 17-ketosteroids were determined by methods previously used in this laboratory.\*

**Subjects** Two normal and 2 diabetic subjects were admitted to the Metabolism Research Laboratory and maintained on constant diets. The 2 normal subjects (R. R., male, age 23, 70 kg, and J. D., male, age 20, 69 kg) received a diet that provided 200 mEq sodium, 113 mEq potassium, 17.3 gm nitrogen, 320 gm carbohydrate, and 2900 calories per day. One diabetic patient (A. R., female, age 62, 87 kg) received 178 gm carbohydrate and 2150 calories per day; the other (R. B., female, age 63, 73 kg) received 150 gm carbohydrate and 1400 calories per day.

Eleven diabetic patients, chosen because they responded poorly to tolbutamide, were studied in the Endocrinology and Metabolism Out Patient Clinic of the University Medical Center.

### Results

**The disappearance of chlorpropamide and tolbutamide from the blood** Measurements of chlorpropamide or tolbutamide disappearance from blood were made after intravenous administration of each compound. The series included observations on 2 normal and 2 diabetic subjects. After an overnight fast each subject received chlorpropamide or tolbutamide sodium\* (30 mg/kg) at 8:00 A.M., and food was withheld for 6 hours.

Data for one of the normal subjects are presented in FIGURE 1. It is seen that the chlorpropamide serum concentration declined from about 21 mg per cent at 15 min to about 18 mg per cent at 6 hours, whereas the tolbutamide serum concentration fell more rapidly from about 20 mg per cent at 15 min to about 8 mg per cent at 6 hours. The serum chlorpropamide

amide. Thus under the circumstances of this experiment, tolbutamide disappeared from the blood approximately 8 times faster than chlorpropamide.

It is of interest to note (FIGURE 1) that there was no significant difference between the two curves at 15 min, 30 min, and 45 min.

**amide** As would be anticipated, there was no correlation between the serum drug levels and the blood glucose levels in these acute (6-hour) experiments. It is likely that under these conditions hypoglycemia was maximally induced when the serum sulfonylurea levels (after the administration of either drug) were above 20 mg per cent for 15 min, 18 mg per cent for 30 min, or 17 mg per cent for 45 min. Reversal of the progressive hypoglycemia at about 45 min was undoubtedly the result of effective counteraction through physiological mechanisms that function to normalize the concentration of blood glucose.

FIGURE 2 shows data for one of the diabetic subjects. This subject had

\* Chlorpropamide was supplied by D. Iczmanski, Chas. Pfizer & Co., Inc. Tolbutamide was supplied by C. J. O'Donovan, The Upjohn Co., Kalamazoo, Mich.

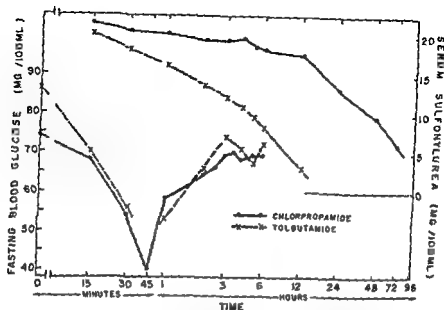


FIGURE 1 Serum sulfonylurea and fasting blood glucose levels after the intravenous administration of chlorpropamide and tolbutamide to a normal subject (J. D., male)

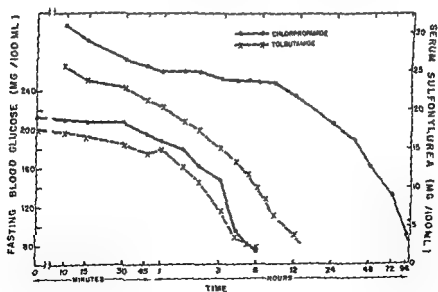


FIGURE 2 Serum sulfonylurea and fasting blood glucose levels after the intravenous administration of chlorpropamide and tolbutamide to a diabetic subject (A. E., female)

somewhat higher serum sulfonylurea levels during the study. The higher serum levels probably resulted from the fact that the dose administered was based upon gross body weight and also from the fact that this subject was moderately obese. Another notable characteristic of the sulfonylurea disappearance curves in this case is the marked difference between the chlorpropamide and tolbutamide serum levels. Since the disappearance rates

In spite of the higher serum levels and the initial differential between the curves the disappearance rates for chlorpropamide and tolbutamide in this diabetic subject were quite similar to those for the previous normal subject (FIGURE 1). It should be noted that this diabetic subject as well as the other one in the series was previously treated with both sulfonylurea compounds. The administration of chlorpropamide to both diabetic subjects was discontinued 9 days before these studies began.

In the diabetic subject (FIGURE 2) there were almost identical progressive reductions in blood sugar after intravenous administration of equal quantities of the 2 drugs. It is impressive that these acute blood glucose responses were remarkably similar in spite of the great difference that had developed in the serum sulfonylurea concentrations by 6 hours. These results are consistent with those obtained in the normal subject and they suggest that the pattern for the blood glucose response was determined at an early time when the serum sulfonylurea concentration for each compound was sufficiently high to induce a maximal response.

In the other 2 subjects, the second normal and the second diabetic, there was also no significant difference in the acute blood glucose responses after intravenous administration of the indicated amounts of chlorpropamide

evaluation is illustrated in FIGURE 3 for one of the diabetic subjects. The disappearance rate is expressed as metabolic half life ( $t_{1/2}$ ) which conventionally is defined as the time required for the serum concentration to decline to one half of a previous level.

It is seen that after chlorpropamide administration at zero time there occurs a very rapid decrease in the serum concentration during the first 10 min (first 2 dots at 5 and 10 min). A rapid decline in serum concentration follows so that the metabolic half life of chlorpropamide during the time interval from 15 through 45 min is calculated to be 2.8 hours. At about the first hour the serum concentration of chlorpropamide stabilizes and remains essentially unchanged up to the sixth hour. During this 5 hour period the half life of chlorpropamide is described of course as infinite. After this plateau phase the half life time fluctuates considerably until about 36 hours when a terminal steady state disappearance begins. During

this phase of terminal steady state disappearance (36 to 144 hours) chlorpropamide is calculated to have a metabolic half life of 35 to 36 hours

The disappearance of tolbutamide (FIGURE 3), after its intravenous administration can be described also in rate phases. The three indicated phases for tolbutamide represent half life times of 3.3, 4.9, and 3.8 hours

The phases which have been described in this subject for chlorpropamide and tolbutamide by half life calculations were also observed in the second diabetic and in both normal subjects. The simplest interpretation which can account for the phases observed after intravenous administration of each compound is (1) an early (0 to 60 min) unstable space distribution, (2) redistribution (1 to 36 hours for chlorpropamide, 1 to 6 hours for tolbutamide) and (3) a stable space distribution (> 36 hours for chlorpropamide

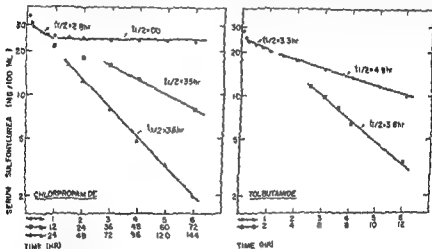


FIGURE 3 Metabolic half life times for chlorpropamide and tolbutamide in a diabetic subject (A.R. female)

> 6 hours for tolbutamide). It would be extremely interesting to make space distribution calculations for strategic timings in such an experimental series as this. Since our studies did not extend to drug balance (excretory studies) this was not possible for these subjects. It was interesting, however, to make initial or zero-time calculations of space distribution in a comparison of the 2 drugs. Extrapolation from the 5 and 10 min points, in the 2 diabetic subjects gave chlorpropamide space volumes of 7.6 and 6.9 l and tolbutamide space volumes of 9.0 and 9.3 l. The mean chlorpropamide and tolbutamide spaces in these 2 diabetics were 9 and 11 per cent of body weight, respectively.

The difference between mean values of 7.3 and 9.2 l for chlorpropamide and tolbutamide spaces respectively would seem highly significant in the 2 subjects. From preliminary studies of deliberate blood sample hemolysis after chlorpropamide and tolbutamide administration it appears that the different space distributions of these 2 drugs may be explainable, to a major

extent on the basis of different concentrations in red cell water. The calculated zero time distribution volumes are probably smaller than the equilibrium distribution volumes and are discussed here only because they point up a difference between the 2 drugs. On the basis of published data by Wick *et al*<sup>7</sup> for tolbutamide in the eviscerated and nephrectomized rabbit, the equilibrium tolbutamide space might be anticipated as 13 to 19 per cent of body weight in man. Zero time distribution calculations have not been made for the 2 normal subjects, since 5- and 10 min points on the sulfonyl-urea disappearance curve, were not obtained.

TABLE 1  
METABOLIC HALF LIFE MEASUREMENTS FOR CHLORPROPAMIDE AND TOLBUTAMIDE  
IN TWO NORMAL AND TWO DIABETIC SUBJECTS

Metabolic half life time (hours)							
Phase	Time interval (hours)	Normal subject			Diabetic subject		
		J D	R R	Mean $\pm$ M D	J R	R D	Mean $\pm$ M D
Chlorpropamide							
A	<1	5.0	2.9	3.0 $\pm$ 0.1	2.8	2.8	2.8 $\pm$ 0.0
B	1 to 6	—	—	—	—	—	—
C	16 to 72	36	33	35 $\pm$ 1	35	37	34 $\pm$ 1
	72 to 120	—	—		36	37	
Postural	24 to 120	35	34	—	32	—	—
Tolbutamide							
A	<1	1.8	1.8	1.8 $\pm$ 0.0	3.3	2.6	3.0 $\pm$ 0.4
B	1 to 6	5.1	6.1	5.6 $\pm$ 0.5	4.9	6.5	5.7 $\pm$ 0.8
C	6 to 12	4.1	4.8	4.6 $\pm$ 0.3	3.8	5.8	4.8 $\pm$ 1.0

Chlorpropamide: tolbutamide disappearance ratio. Normal subjects  $35/46 = 7.6$   
diabetic subjects  $35/48 = 7.3$

The disappearance data for all 4 subjects in the series are summarized in TABLE 1. A, B, and C represent the 3 phases previously described for intravenous chlorpropamide and tolbutamide in conjunction with FIGURE 1. There are probably no significant differences in the metabolic half life times between the 2 normal and the 2 diabetic subjects for any of the 3 phases with each of the drugs, with the possible exception of the tolbutamide phase A.

Half life times calculated after the cessation of oral (postoral) chlorpropamide administration are given in TABLE 3 for 3 of the 4 subjects as well.

The phase C and postoral metabolic half-life times, which represent terminal steady-state disappearance and a wide range of serum concentrations,



were used to arrive at a comparison of the rates of disappearance of these 2 drugs from the blood. This comparison is given as a ratio calculation below TABLE 3 and indicates that tolbutamide disappears from the blood from 7 to 8 times faster than chlorpropamide.

*The relative potency of chlorpropamide and tolbutamide.* It was clear that acute studies and classic dose response studies would yield information of limited value in a potency comparison of chlorpropamide and tolbutamide, especially if one attempted to apply such information in terms of long term oral therapy. On the other hand, it appeared as though meaningful data

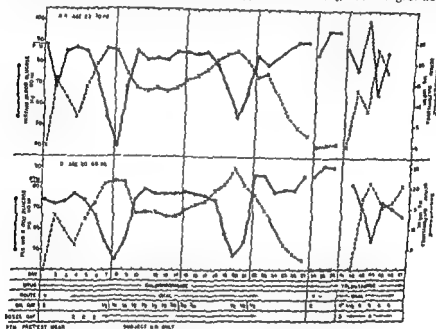


FIGURE 4. Fasting blood glucose and fasting serum sulfonylurea levels in 2 normal men during the administration of chlorpropamide and tolbutamide.

could be derived from long term comparisons of the 2 drugs at various dosage levels and from a separation of the classic dose response study into 2 distinct components: the relation of dose to drug blood level and the relation of drug blood level to blood glucose response. Such an approach would take into account the facts that (1) the 2 drugs had markedly different half life times, and (2) the 2 drugs probably had different spaces of distribution. With these considerations in mind, 2 normal men were studied for a period of about 4 weeks on an identical schedule of changing doses of oral chlorpropamide (FIGURE 4). During this period the men were carried through 2 cycles of symptomatic hypoglycemia. In a subsequent period they were observed during episodes of tolbutamide induced hypoglycemia. Much larger oral dosages of tolbutamide (at least 6 times larger) were required to produce comparable hypoglycemia and comparable serum sul

fonylurea levels. Daily measurements of the fasting blood glucose and the fasting serum sulfonylurea levels during oral administration of the 2 drugs revealed a consistent pattern.

In FIGURE 5 the fasting serum sulfonylurea levels are plotted against changes in the fasting blood glucose level for the 2 normal men. Standard

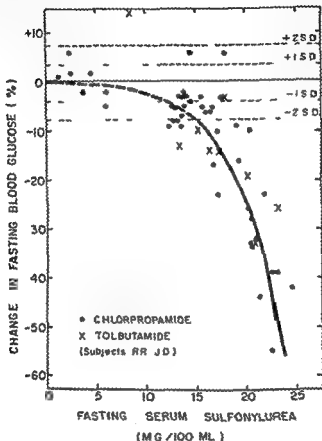


FIGURE 5. Correlation between change in fasting blood glucose and fasting serum sulfonylurea levels in 2 normal subjects.

deviations relate to both men for daily fasting blood glucose measurements during an 8-day pretest period. The curve was originally constructed in the absence of the tolbutamide points with reference only to the chlorpropamide points. It is apparent, however, that when the tolbutamide points are included as they are in this figure they fit the previously constructed curve for chlorpropamide. These results indicate that within the standardizations and extendiveness of these observations chlorpropamide

a decrease of one standard deviation in the fasting blood glucose in these normal men. A decrease of twice the standard deviation in the fasting blood glucose resulted from a serum sulfonylurea level of about 15 mg per

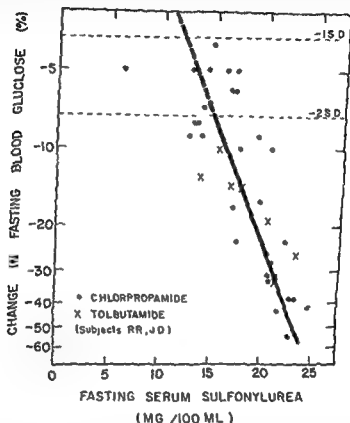


FIGURE 6 Correlation between change in fasting blood glucose and fasting serum sulfonylurea levels in 2 normal subjects (semilogarithmic presentation)

cent. All sulfonylurea levels above 18.5 mg per cent produced decreases in the fasting blood glucose of at least twice the standard deviation. Within the range of significant measurements, increments in serum sulfonylurea concentration of approximately 3 mg per cent double the effect on the fasting blood glucose.

The data from FIGURE 5 are reproduced in FIGURE 6 with semilogarithmic coordinates. Under this circumstance the relationship between the fasting serum sulfonylurea level and the change in the fasting blood glucose is represented by a straight line.

Since chlorpropamide and tolbutamide on the basis of their serum concentrations are approximately equally active, and since tolbutamide disappears from the blood approximately 8 times faster than chlorpropamide it follows that chlorpropamide has the potentiality of being approximately 8 times more effective than tolbutamide on a weight basis, when the 2 drugs are used as oral therapeutic agents.

It was of interest to obtain information on how the serum chlorpropamide concentration varies during a 24 hour period when 500 to 750 mg is administered each morning with breakfast (TABLE 4). Although the 2 subjects had

TABLE 4  
VARIATION OF SERUM CHLORPROPAMIDE LEVELS DURING THE DAY AFTER SINGLE ORAL MORNING DOSES OF THE DRUG IN 2 NORMAL MEN

Subject	Day*	Chlorpropamide dose (gm./day)†	Time	Serum chlorpropamide (mg./100 ml.)	Per cent of initial fasting level
R. R. (70 kg.)	15	0.75	8:00 A.M.	15.7	100
			10:00 A.M.	23.5	150
			12:00 P.M.	24.6	157
			2:00 P.M.	21.4	136
	16	0.50	8:00 A.M.	16.8	107
			8:00 A.M.	22.7	100
			3:00 P.M.	27.2	120
	21	0.50	8:00 A.M.	20.3	89
			8:00 A.M.	20.1	100
			5:00 P.M.	24.6	121
	22		8:00 A.M.	16.5	81
J. D. (69 kg.)	15	0.75	8:00 A.M.	14.6	100
			10:00 A.M.	16.0	110
			12:00 P.M.	18.0	123
			2:00 P.M.	17.0	116
	16	0.50	8:00 A.M.	15.8	108
			8:00 A.M.	24.6	100
			4:00 P.M.	26.8	109
	21	0.50	8:00 A.M.	20.6	84
			8:00 A.M.	20.6	100
			5:00 P.M.	21.2	103
	22		8:00 A.M.	18.2	88

\* Days correspond with those in FIGURE 4.

† Total dose taken shortly after 8:00 A.M. with breakfast.

very similar fasting serum chlorpropamide concentrations on the same drug dosages they showed somewhat different but quite consistent variations in serum levels during the intervals studied. The subjects developed maximum serum chlorpropamide concentrations of 123 and 157 per cent of the fasting levels about 4 hours after an oral dose at 8:00 A.M. The serum levels at 5:00 P.M. were 103 and 121 per cent of the morning fasting levels even when there was a moderate decline in the fasting serum level the following morning.

Thus, a single dose of chlorpropamide at 8 00 A M would seem to provide elevated serum sulfonylurea levels (that is, relative to the fasting level) during the absorptive portion of the day (about 8 00 A M to 8 00 P M) and to allow the serum level to decline to a near fasting level early in the usually non absorptive portion (about 8 00 P M to 8 00 A M) of the day. This feature, which is provided by the long metabolic half life time of chlorpropamide, may prove to be advantageous therapeutically.

*Clinical studies* Eleven diabetic patients who responded poorly to tolbutamide were treated with chlorpropamide. All responded better to chlorpropamide at considerably lower daily dosages in most cases.

The comparative response of the 11 diabetic patients to the 2 compounds is illustrated in TABLE 5. Each patient received each substance, at the indicated dosages, for at least 3 days and usually for 1 week prior to the determinations. Complete comparative data were obtained for the first 3 patients.

TABLE 5  
COMPARATIVE RESPONSE OF PATIENTS WITH DIABETES MELLITUS TO ORAL  
TOLBUTAMIDE AND CHLORPROPAMIDE THERAPY

Patient	Tolbutamide			Chlorpropamide		
	Dosage (gm./day)	Fasting blood glucose (mg./100 ml.)	Fasting serum tolbutamide (mg./100 ml.)	Dosage (gm./day)	Fasting blood glucose (mg./100 ml.)	Fasting serum chlorpropamide (mg./100 ml.)
A. R. (95 kg.)	3.0	191	5.3	0.75	138	14.2
E. E. (74 kg.)	1.5	183	6.1	0.5	86	19.5
H. H. (60 kg.)	3.0	176	4.6	1.0	88	29.3
R. B. (73 kg.)	2.0	150	—	1.0	136	23.0
A. C. (58 kg.)	2.0	210	—	2.0	146	—
P. P. (55 kg.)	2.0	168	—	1.0	131	16.1
K. M. (72 kg.)	1.0	155	—	0.5	81	22.2
M. J. (107 kg.)	3.0	227	—	1.5	155	36.3
M. R. (88 kg.)	1.5	153	—	0.5	109	10.2
P. S. (41 kg.)	1.5	210	—	1.0	139	35.5
N. M. (75 kg.)	1.0	131	—	0.25	99	7.5

listed. The decreases in the fasting blood glucose levels experienced by these 3 patients on the indicated tolbutamide dosages were slight. The relatively low fasting serum tolbutamide levels are to be noted. In the same 3 patients considerably lower dosages of chlorpropamide produced higher serum levels of the drug and, concurrently, more intense decreases of the fasting blood glucose levels. The same pattern of response described for the first 3 patients is characteristic of the other 8, as well.

... consistent with the  
...  
... to its longer metabolic half life ...



An example of the difference in response to the 2 drugs is illustrated in FIGURE 7

*Metabolic balance studies*: Two normal men were studied for a period of about 4 weeks on an identical schedule of changing doses of chlorpropamide. The balance and renal excretion studies (TABLE 6) during chlorpropamide administration yielded results similar to those previously published from this laboratory for tolbutamide and carbutamide.<sup>6</sup>

There was no effect on urinary excretion of 17 ketost

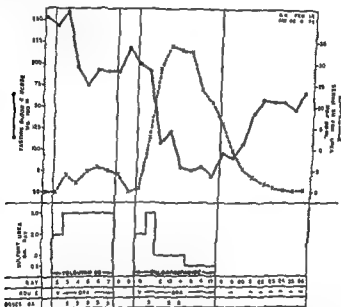


FIGURE 7 Fasting blood glucose and fasting serum sulfonylurea levels in a diabetic subject during the administration of tolbutamide and chlorpropamide

days when severe symptomatic hypoglycemia had been produced, urinary excretion of nitrogen and electrolytes was significantly decreased and the excretion of 17 hydroxycorticoids was increased. Serum levels of sodium, potassium, and chloride likewise were not affected.

#### Summary and Conclusions

- (1) A single analytical method has been found to perform well for the

study of normal men and diabetic subjects on chlorpropamide

(4) Chlorpropamide, when compared with tolbutamide on a weight basis, exhibits a markedly greater clinical effectiveness

(5) The greater clinical efficacy observed with chlorpropamide seems adequately explainable on the basis of the capacity of the drug to produce and maintain higher serum levels on a given dosage schedule

### References

- 1 FORIST A A W L MILLER JR J BRAKE & W A STRICK 1957 Determination of plasma levels of tolbutamide *Proc Soc Exptl Biol Med* **96** 189
- 2 SPINGLER H & F KAISER 1956 Die Bestimmung von N (4 Methyl benzolsulfonyl) N butyl harnstoff in Serum *Arzneimittel Forsch* **6** 760
- 3 SPINGLER H 1957 Über eine Möglichkeit zur colorimetrischen Bestimmung von N (4 Methyl benzolsulfonyl) N butyl Harnstoff in Serum *Klin Wochschr* **35** 533
- 4 BLADH G & A NORDEY 1958 A method for determining tolbutamide in human blood serum *Acta Pharmacol Toxicol* **14** 188
- 5 LOTIS L H S S FAJANS J W COVY W A STRICK J B WRIGHT & J L JOHNSON 1956 The structure of a urinary excretion product of 1 butyl 3 p tolylsulfonylurea *J Am Chem Soc* **78** 5701
- 6 FAJANS S S L H LOUIS H S SELTZER H M JOHNSON R D GITTNER A R HENNEY B L WACHENBERG J P ACKERMAN & J W COVY 1956 Metabolic effects of arylsulfonylurea compounds in normal men and in diabetic subjects *Metabolism* **5** 820
- 7 WICK A N B BRITTON & R GRABOWSKI 1956 The action of Ornase in extra hepatic tissues *Metabolism* **5** 739



# A COMPARISON OF THE ACUTE HYPOGLYCEMIC POTENCIES OF TOLBUTAMIDE AND CHLORPROPAMIDE\*

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Comparisons of the clinical effectiveness of tolbutamide and chlorpropamide during their chronic administration to diabetic patients have indicated that smaller doses of chlorpropamide are required to produce a similar lowering of the blood glucose concentration. At least part of this greater clinical potency of chlorpropamide as compared to tolbutamide is explained by its slower rate of disappearance from the body. In the current studies the inherent hypoglycemic potencies of tolbutamide and chlorpropamide have been compared in terms of the acute decreases in blood glucose produced by the intravenous administration of equal doses of the two drugs to the same diabetic patients. Use of the intravenous rather than the oral route of administration avoided possible differences in the rates of gastrointestinal absorption of the two compounds, and the acute nature of the study permitted a comparison of the effects of the drugs before the difference in their half-lives had become manifest in significantly different plasma drug levels.

Each patient was tested twice, once with tolbutamide† and on another occasion with an equal dose of chlorpropamide‡. The patients were free of the influence of any hypoglycemic agent—that is, they had received no long-acting insulin for at least 48 hours, no regular insulin for 15 hours, no tolbutamide for 15 hours, and no chlorpropamide for at least 96 hours before any of the tests. Preceding the tests the patients rested in bed after an overnight fast. A 5 per cent aqueous solution of the sodium salt of the drug was injected into a peripheral vein within 1 min. Blood samples were obtained through

... 15 min intervals, after blood glucose opamide and tolbutamide by the method of Looalan and Wagues. The statistical significance of differences between responses to the two drugs was assessed by analysis of the mean differences of paired observations.

As shown in TABLE 1, equal doses of tolbutamide and chlorpropamide produced similar decreases in the blood glucose concentration during the 3 hours after drug administration. The differences between these declines were not statistically significant. The plasma concentrations of the drugs are shown in TABLE 2. Although at 2 and 3 hours the average tolbutamide levels were slightly less than the chlorpropamide levels, again the differences were not statistically significant.

\* Diabetes Research Program of  
Research Grant A 1317 from the  
Public Health Service, Bethesda  
† Related Metabolic Diseases  
Foundation, Inc., New York

† Supplied by The Upjohn Company, Kalamazoo, Mich.  
‡ Supplied by Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

TABLE 1  
MEAN DECREASES IN BLOOD GLUCOSE CONCENTRATION PRODUCED BY THE INTRAVENOUS ADMINISTRATION OF EQUAL QUANTITIES OF SODIUM TOLBUTAMIDE (T) OR SODIUM CHLORPROPAMIDE (C) TO THE SAME DIABETIC PATIENTS

Dose of drug (gm)	No of subjects	Drug administered	Fasting blood sugar (mg/100 ml)	Blood glucose decrease (mg/100 ml)					
				Minutes after administration					
				30	60	90	120	150	180
1.0	8	T	187	11.5	20.2	32.8	41.1	—	55.4
		C	173	9.7	23.2	30.5	41.3	—	54.7
		TC*	—	18 ± 8.4	-3.0 ± 4.7	2.3 ± 5.4	-0.2 ± 6.2	—	-0.3 ± 5.7
0.5	4	T	213	—	15.0	17.8	27.7	—	48.0†
		C	206	13.5	22.0	32.0	38.2	—	59.5†
		TC*	—	—	7.0 ± 9.5	-14.2 ± 7.0	-10.5 ± 7.8	—	-11.5†
0.25	3	T	134	11.3	19.3	29.7	30.6	—	26.5
		C	131	—	15.3	19.3	21.0	—	27.0
		TC*	—	—	4.0	10.4	7.6	—	-0.5
0.125	2	T	202	6.5	15.0	23.5	31.0	33.5	19.0
		C	202	4.0	—	15.0	20.5	27.0	38.0
		TC*	—	2.5	—	8.5	10.5	6.5	1.0

\* Mean difference ± one standard error between blood glucose decreases after tolbutamide and decreases after chlorpropamide

† Data from only 2 of the 4 subjects

TABLE 2  
MEAN PLASMA CONCENTRATIONS OF TOBUZAMIDE (T) AND CHLORPROPAMIDE (C)  
AFTER INTRAVEINOUS ADMINISTRATION OF EQUAL QUANTITIES OF THE SODIUM  
SALTS OF THE DRUGS TO THE SAME DIABETIC PATIENTS

Dose of drug (mg)	No of subjects	Drug administered	Concentration of drug in plasma (mg /100 ml)		
			Minutes after administration		
			60	120	180
1.0	8	T C T C*	9.9 8.8 1.1 ± 1.2	7.8 8.6 ~0.8 ± 1.8	6.4 8.1 -1.7 ± 1.1
0.5	4	T C T C*	5.8 7.2 1.4 ± 1.3	4.5 5.0 ~0.5 ± 0.2	3.8† 4.8† —
0.25	3	T C T C*	3.0 2.5 0.5	2.0 2.4 ~0.4	1.5 1.9 ~0.4
0.125	2	T C T C*	2.8† 1.2† 1.6	0.7 1.1 ~0.4	0.5 — —

\* Mean difference + one standard error between plasma concentrations of tolbutam de and chlorpromamide

† Only 2 values included

† One of samples obtained at 90 min

§ Both samples obtained at 90 min

Thus at similar blood levels the two drugs produced a similar fall in blood glucose during the first three hours after their intravenous administration to diabetic patients. These data cannot be applied directly to the chronic administration of the drugs however, Knauff *et al*<sup>2</sup> found during long term administration of tolbutamide and chlorpropamide that the degree of fasting hypoglycemia was a function of the fasting sulfonylurea level and that the same relationship applied to both drugs. These findings suggest — \* for chlorpropamide as compared to tolbutamide rather than to any of the two drugs.

## References

- 1 SOMOGI M 1945
- 2 TOOLAN T J & R I  
amide and its deter
- 3 KNAUFF R F S S F  
chloropropanide in not  
74(3) 603-617

### Part III : Clinicometabolic Studies

## STUDIES WITH CHLORPROPAMIDE IN DIABETIC PATIENTS\*

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The clinical trials of chlorpropamide reported here were begun in April 1958; our experience to date therefore extends over only 5 months. The studies were initiated with the following four objectives in mind: first to determine the effectiveness of the drug as a hypoglycemic agent in the management of diabetes; second to observe serum levels of the compound in patients under control; third to determine whether or not long term effectiveness could be predicted by the blood sugar response in a single dose test; and finally to study the possible toxicity of the drug by performing serial liver and kidney function tests and hematological studies.

### *Clinical Material and Methods*

Seventy-eight patients with proved diabetes, selected primarily on the likelihood of responsiveness to sulfonylurea compounds, have been given chlorpropamide to date. The group consisted of 48 females and 30 males. The ages of this group varied between 35 and 83 years and all but 10 were above 50 years of age. The age at onset of diabetes varied from 23 to 77 years; in 10 patients this had been below the age of 40 years. The duration of diabetes varied from a few weeks to 21 years; in 60 patients this was more than 1 year.

In all cases but one the patients were hospitalized and maintained on weighed diets. The total amount of sugar excreted in the urine was determined for each 24 hour period. The blood sugar was determined frequently, usually 3 times daily, before breakfast at 11 A.M. and at 3 P.M. The majority of the patients were ambulatory. The diets provided from 130 to 200 gm of carbohydrate daily, with most in the range of 150 to 170 gm. The caloric content varied from 1000 to 2000 calories daily; often this was low so as to favor weight reduction. In all but 21 patients the insulin requirement had been established either by long term usage or during a brief period of regulation prior to trial of the oral hypoglycemic agent. In the 57 patients whose insulin requirement was known, this was 40 units or below in 47 patients and 20 units or below in 22 patients.

Blood sugar determinations were carried out by the Somogyi-Nelson procedure. Most blood sugar levels before breakfast were determined on venous blood; most other samples were capillary. For the determination

\* The work reported in this paper was supported in part by grants from Chas. Pfizer & Co. Inc., Brooklyn, N. Y., and The Upjohn Company, Kalamazoo, Mich.

† Research Fellow.

of the level of chlorpropamide we used the method of Spingler<sup>1</sup> as modified by R. H. Carmichael. In this procedure serum is extracted with isoamyl acetate and treated with 1 fluoro-2,4-dinitrobenzene and the resulting color measured in the Beckman spectrophotometer at 345 m $\mu$ . Prior to the beginning of therapy most patients had base line liver function studies including

function studies are being done after each 3 months of treatment with the drug.

**Sulfonylurea response test** Prior to long term trial with chlorpropamide a single dose response test was done. In general the procedure used was the same as that previously described in studies with carbutamide and tolbutamide.<sup>2</sup> The test was carried out as follows: (1) long acting insulin was omitted for 24 hours prior to the test day with crystalline insulin being substituted where necessary, and (2) at 8 A.M. on the day of the test 1.0 gm of chlorpropamide was given orally after an overnight fast and glucose determinations were done on 3 blood samples: (a) fasting before chlorpropamide, (b) fasting 12 noon and (c) at 3 P.M. (about 2½ hours after a regular noon meal).

The maintenance dose in almost all patients was either 0.25 or 0.50 gm/day. A few patients received 1 gm/day for short periods during the early period of the study.

### Results

**Response tests** The response test was carried out in 71 patients. In 37 a fall in blood sugar of 20 per cent or more occurred at both 4 and 7 hours.

F<sub>1</sub>

7

periods varying from a few days to 140 days. Initially 16 per cent of the dose for periods longer than one month and 48 for more than 10 days.

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t  
reasons poor control on maintenance 12 medical reasons 11  
nonmedical reasons 11

The average  
test dose was

Minor toxic effects consisted of minimal gastrointestinal symptoms.

2 patients and a skin rash in another. One patient developed jaundice after 3 weeks of therapy with 0.50 gm/day. He was hospitalized, and initial laboratory studies showed the following results: bilirubin, total, 3.9 and direct, 3.0 mg/100 ml, Bromsulphalein retention, 49 per cent at 45 min, alkaline phosphatase, 19.2 Bodansky units, thymol turbidity, 0.3 units, and cephalin and thymol flocculations negative. The differential white blood cell count showed an eosinophilia of 10 per cent, falling to 1 per cent after 12 days. Liver biopsy showed canalicular bile stasis, but no evidence of hepatocellular damage. The patient improved with conservative therapy, and the last follow up 2 months later showed the following laboratory results: bromsulphalein retention, 9 per cent in 45 min, alkaline phosphatase, 4.7 Bodansky units, thymol turbidity, 0.8 units, and thymol flocculation negative.

### Discussion

Of the 50 patients who showed a fall of more than 20 per cent in blood glucose in the 4 hour sample of the response test, 41 achieved good control. Of the 31 patients showing a fall of 20 per cent or more in both 4- and 7 hour samples, 26 achieved good control. These results in conjunction with the finding of an adequate serum level of chlorpropamide at 4 hours suggest that the response test carried over 7 hours has no advantage over the 4 hour test. Eighty per cent of patients having a satisfactory response with either test are likely to have their diabetes controlled. Of the 13 patients who had less than a 20 per cent fall at 4 hours, 7 achieved good control, 2 fair control, and 4 poor control. It thus seems that a poor response test does not preclude subsequent control of diabetes with chlorpropamide.

The serum chlorpropamide determinations suggest that adequate levels are attained within 4 hours and maintained over 24 hours after an initial dose of 1 gm, and throughout longer periods with doses of 0.25 to 0.50 gm/day.

In evaluating the effectiveness of chlorpropamide in controlling diabetes, the role of diet must be considered. As most of our patients were obese, their diets were restricted in calories as well as carbohydrate content. The average weight loss of our patients during this study period was 2.9 kg, and in 11 patients chlorpropamide could be discontinued and control maintained with diet alone.

The only serious toxic manifestation in our group of patients was the occurrence of jaundice in a 54-year-old man. The clinical picture, the laboratory data, and the histological appearance of a punch biopsy of the liver were all similar to findings in jaundice produced by chlorpromazine and methyl testosterone. It is noteworthy, however, that our patient had a past and family history of jaundice, the nature of which was not determined.

### Summary and Conclusions

(1) Seventy-eight patients, selected mainly on the likelihood of responsiveness to sulfonurea compounds, were given chlorpropamide. All but 10 were above 50 years of age, and the onset of diabetes occurred before the age of 40 in 10 patients.

(2) In 71 patients the response of the blood sugar to 1 gm of chlorpropamide was determined at 4 and 7 hours. Eighty per cent of the patients who had a fall of 20 per cent or more at 4 hours subsequently achieved good control of hypoglycemia and glycosuria with maintenance doses. Of the 72 patients who received the drug for a period of up to 140 days 68 per cent achieved good control, 15 per cent fair control, and 14 per cent poor control.

(3) Serum chlorpropamide levels ranged between 3 and 32 mg per 100 ml, with an average of 10 mg per 100 ml.

(4) One patient with a past and family history of jaundice developed an obstructive type of jaundice 3 weeks after onset of therapy. There was complete recovery. Two patients had minor gastrointestinal symptoms and 1 patient developed a skin rash.

(5) These preliminary studies indicate that, in selected patients, chlorpropamide is an effective hypoglycemic agent. Wider experience with the drug is needed to evaluate its long term usefulness and incidence of untoward effects.

### References

- 1 SPINGLER H. 1957. Über eine Möglichkeit zur colorimetrischen Bestimmung von N-(4-Methyl benzolsulfonyl)-N-butyl Harnstoff im Serum. *Klin Wochschr* 35: 533.
- 2 MARBLE A. 1958. Oral hypoglycemic agents in the management of diabetes. *Med Clin N Am* 42: 1163.

## USE OF CHLORPROPAMIDE IN DIABETES MELLITUS IN USUAL DIABETIC PATIENTS AND IN PRIMARY AND SECONDARY TOLBUTAMIDE FAILURES\*

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In a preliminary report<sup>1</sup> on management of diabetes with chlorpropamide (Diabinese†) successful control was noted in 58 per cent of 45 patients studied. Further observations were made in 70 cases for periods up to 8 months. We were particularly interested in observing the effectiveness of chlorpropamide in a group of patients who were primarily unresponsive to tolbutamide (within the first month of treatment) and in a larger series of cases classified as secondary tolbutamide failures—that is, patients in whom diabetes had slipped out of control after a variable period of satisfactory management with tolbutamide.

### *Method of Study*

Diabetic patients who were taking insulin, an occasional patient not controlled with diet alone, and those not responding to tolbutamide were used in this investigation. The first few patients were hospitalized, but later the drug was given to clinic and ambulatory private patients. Diet was prescribed according to optimal caloric requirements.

In the hospital, fractional urinalyses for sugar and acetone were made by the ward nurses before meals and at bedtime. Each test showing more than a trace of sugar (Clinitest‡ modification of Benedict's test) was covered by an injection of regular insulin ranging in dosage from 5 to 20 U depending upon the percentage of sugar present. In this way we were able to prevent ketosis and at the same time observe the degree of effectiveness of chlorpropamide. Daily fasting blood sugar, an occasional blood sugar after a meal, and 24 hour quantitative urine glucose determinations were made as added parameters of control.<sup>2</sup>

3 days indicated that these requirements had been attained, patients were given a supply of medication and discharged for follow up in the outpatient clinic. Particular stress was laid on the need for frequent urine tests at

Inc. Brooklyn N Y

† The Clinitest apparatus and tablets are manufactured by The Ames Co., Inc. Elkhart Ind.



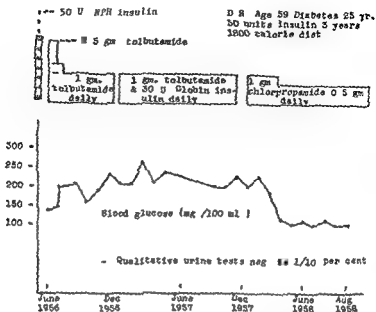


FIGURE 3 Good control with chlorpropamide after tolbutamide secondary failure

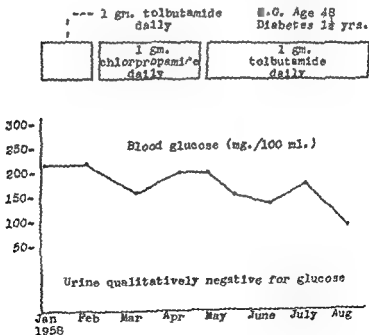


FIGURE 4 Good response to a second trial of tolbutamide after discontinuing chlorpropamide because of disagreeable taste

quate control, as measured by the criteria described above. Six of the 28 patients who failed to respond to chlorpropamide were brittle type diabetics requiring large doses of insulin and having a pronounced tendency to acidosis. For reasons such as fear of injections or recentness of onset of diabetes, 4 patients had not previously taken insulin. These cases were easily controlled with chlorpropamide. Three patients who had been taking insulin for more than 3 years remained well controlled after chlorpropamide was discontinued. However, one of these (FIGURE 1) developed hyperglycemia without glycosuria after 2 months without medication. This was relieved by a small daily dose of 0.25 gm chlorpropamide. Only 2 of the 10 cases classified as primary tolbutamide failures responded well to chlorpropamide. One of these is shown graphically in FIGURE 2. Our experience with secondary or late tolbutamide failures was much better. Twenty of the 31 (64 per cent) patients in this group were managed satisfactorily with chlorpropamide. A representative case is shown in FIGURE 3. Another noteworthy feature was a good response to a second course of tolbutamide in 6 patients after chlorpropamide was stopped because of side effects (FIGURE 4). We suspect that the patients who responded to a second course of tolbutamide did so because of a renewed respect for adherence to diet.

### Toxicity

Side reactions to chlorpropamide are listed in TABLE 2. Gastrointestinal symptoms of anorexia, nausea, vomiting and abdominal discomfort were the most prominent. Chest pain severe enough to warrant cardiovascular investigation was noted in 8 patients. Electrocardiograms in all of these showed evidence of coronary artery disease and two of these later developed myocardial infarction. Chlorpropamide was continued in 2 of these cases and the chest pain gradually subsided. In fact 2 patients were given chlorpropamide with good effect soon after suffering an acute myocardial infarct. Another prominent complaint was weakness, usually of legs, arms and abdominal muscles. Two of the 7 patients with this complaint had

TABLE 2  
SIDE REACTIONS TO CHLORPROPAMIDE

	No. of patients*
Anorexia, nausea, vomiting, abdominal discomfort	9
Chest pain	8
Muscle weakness	7
11	4
4	4
1	3
1	1

\* Of a total number of 10 patients treated

responded as far as diabetic control was concerned, but the symptom was so severe that it became necessary to discontinue the drug. Serum potassium levels determined before and during treatment with chlorpropamide showed no diminution.

Hypoglycemic symptoms (anxiety, hunger, sweating) were noted in 4 patients. These never progressed to coma and disappeared promptly with administration of sweet drinks and reduced dosage.



FIGURE 5. Maculopapular rash on face, neck, and arms following administration of chlorpropamide.

Four patients with itching, two with associated maculopapular rash of face, arms, and legs were noted. One of these (FIGURE 5) was severe enough to warrant discontinuing medication, but the others responded to antihistamine preparations without discontinuing chlorpropamide.

Three patients had an excellent response to a daily dose of 1.0 gm, but asked for a change of medication because of a disagreeable taste sensation. This was described as a "sick, soot taste, even in the lips." In retrospect we believe this could have been avoided by starting with a smaller daily dose of 0.5 gm.

One case of leukopenia with a white blood cell count of 2500 was noted. This was not permanent; when medication was discontinued, the count

promptly returned to normal. The same patient complained of loss of hair after chlorpropamide had been discontinued. Examination failed to substantiate this disorder.

A depression of thyroid uptake of radioiodine was noted in 5 patients. The range of lowering was 4 to 15 per cent. There were no associated symptoms of hypothyroidism and this reaction was not considered of clinical significance.

No unusual effects on kidneys or liver have been noted to date. The longest period of observation has been 8 months.

One patient died of coronary thrombosis after having been controlled with chlorpropamide for 4 months. He was known to have malignant hypertension and his death was in no way attributable to the drug.

#### Conclusions

Of 70 diabetics studied, 60 per cent were controlled satisfactorily with chlorpropamide.

The most easily controlled were middle aged patients with relatively

Toxicity to chlorpropamide is related partly to dosage. Gastrointestinal symptoms, chest pain, and muscular weakness were noted in one third of the patients studied. Occasionally these were relieved by reduction in dose of the drug.

In most cases a smaller dose of chlorpropamide gave as good if not better control than tolbutamide.

Untable diabetics of any age are poor candidates for control with chlorpropamide.

#### References

1. SUGAR, S. J., & L. J. THOMAS & S. TATLER. 1958. Management of diabetes mellitus with chlorpropamide. *Med Ann Dist Columbia* 27: 445.
2. NELSON, N. 1944. Photometric adaptation of Somogyi method for determination of glucose. *J Biol Chem* 153: 375.

## A LABORATORY AND CLINICAL STUDY OF CHLORPROPAMIDE IN AMBULATORY DIABETICS

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Pharmacological studies have indicated that chlorpropamide is a potent hypoglycemic agent that is relatively nontoxic. With a background of this knowledge, clinical application of the drug was instituted in the Diabetes Clinic of the Long Island College Hospital 4 months ago. Six elderly mild diabetics were given 1 tablet (500 mg) of chlorpropamide 3 times a day (a total of 1.5 gm) on the first day and 1 tablet twice daily (a total of 1 gm) thereafter. Four of the patients complained of severe muscle weakness and dizziness leading to discontinuance of the drug. This initial experience prompted a more detailed investigation of the pharmacological properties of chlorpropamide in humans, since the experience obtained with other oral hypoglycemic agents did not seem to apply to this more potent agent.

A method of estimating chlorpropamide, as developed by the Research Department of Chas. Pfizer & Co., Inc., Brooklyn, N. Y., as described elsewhere in this monograph by Toolan and Wagner, was employed to study the level of the drug in sera of patients following single doses as well as during long term administration of the medication. The results of these investigations are the subject of the present work. Clinical observations concerning the efficacy of the medication observed while treating ambulatory diabetics will be discussed elsewhere.

### *Serum Levels Following Single Doses Given Orally or Intravenously*

Serum estimations of the serum level of chlorpropamide as well as blood sugar were made at 20 min. intervals for 4 hours after the administration of single doses of the medication. TABLE 1 shows a summary of the serum drug levels found after a 0.5 gm. dose was given orally and after an equivalent dose of the drug was given intravenously as the sodium salt. The intravenous injections were accomplished over a 2 min. period. Both diabetic and non-diabetic patients were studied. Two facts are to be noted. First the drug was well absorbed, although somewhat slowly when given orally. Two hours after oral administration high serum levels were obtained and within 4 hours the concentrations in serum were in a range similar to those obtained after the intravenous injection. Second, an appreciable quantity of the medication was still found in the serum 24 hours after its administration.

These observations concerning the good absorption and prolonged retention of the drug in serum were again found when 1 gm. doses of chlorpropamide were given orally and intravenously (TABLE 2, A and B). In this series oral administration was found to yield serum values at 4 and 24 hours that were higher than those obtained after a similar dose had been given intravenously.

This finding suggested that slow absorption from the intestinal tract might be a factor causing the prolonged elevation of drug levels in the blood. As can be seen in TABLE 2 (C and D), after giving single oral doses of 1.5 and 2 gm, proportionate increases in serum chlorpropamide levels were not obtained in 4 hours in most experiments. The larger doses led to higher concentrations in the serum after 24 hours.

TABLE 1  
SERUM LEVELS OF CHLORPROPAMIDE AFTER SINGLE DOSE OF 0.5 GM

Case	Diabetic Non-diabetic	Body habitus†	Serum level (mg %) at						
			20 min	40 min	1 hour	2 hours	3 hours	4 hours	24 hours
A—Intravenous									
C.H.S.	ND	O	7.9	6.7	6.3	5.6	5.8	5.0	3.1
J.J.S.	ND	O	4.5	4.0	4.8	4.0	3.6	3.8	1.6
U.C.S.	ND	T	6.4	6.8	7.2	6.2	5.6	5.6	2.4
B.C.H.	ND	T	7.9	6.3	U	6.0	6.0	5.8	3.0
C.H.D.	D	M	7.2	7.0	3.7	6.4	3.7	2.2	—
D.H.R.	D	O	3.3	4.3	4.3	6.1	6.2	5.3	4.7
C.R.T.	D	O	7.2	4.5	4.3	—	3.9	4.3	0.3
D.T.I.	D	N	6.9	6.2	5.6	5.2	5.6	5.1	1.9
Q	D	O	6.2	5.5	3.3	4.9	4.6	4.4	1.7
			Mean 6.39	5.70	5.63	5.55	5.44	4.61	2.34
			S.D. 1.55	1.17	1.06	0.81	0.83	1.11	1.30
B—Orally									
U.C.A.	ND	O	2.4	2.6	3.0	7.1	6.5	5.8	—
D.C.A.	ND	O	0	0.9	1.7	2.3	3.3	3.6	2.5
C.C.A.	D	M	8	1.8	2.2	2.5	—	4.4	2.5
	D	M	8	1.0	1.4	4.8	5.7	5.8	2.0
			Mean 0.6	1.58	2.08	4.23	5.10	4.70	2.33
			S.D. 1.08	.79	.70	2.20	1.59	0.93	0.24

\* Symbols: D = diabetic \ D = nondiabetic

† Symbols: O = obese T = thin M = medium

The variations in blood levels obtained in different patients after the same dose of chlorpropamide had been given (TABLES 1 and 2) could not be related to differences in absorption since it occurred after intravenous administration as well. The size of the patient influenced this to some extent. In TABLE 2

of the drug assuming that the medication remained in the extracellular space. In obese patients the space was usually larger (25 to 27 per cent) and in thin patients such calculations found it usually smaller (16 to 18 per cent).

TABLE 2  
SERUM LEVELS OF CHLORPROPAMIDE AFTER SINGLE DOSES

Case	Diabetic Non diabetic*	Body habitus†	Serum level (mg %)‡						
			20 min	40 min	1 hour	2 hours	3 hours	4 hours	24 hours
A—Intravenous—10 gm									
M V	N D	O	4.5	5.4	4.3	5.1	—	—	—
T F	N D	T	14.5	13.1	12.0	11.7	11.4	10.3	—
R A	N D	T	5.3	5.4	5.4	4.8	4.6	3.2	2.4
M C	D	T	6.4	13.7	10.5	10.3	9.8	9.8	3.6
W	D	T	12.5	10.2	10.6	8.9	—	8.5	1.3
			Mean 8.6	9.6	8.6	8.2	8.6	8.0	2.4
B—Orally—10 gm									
S B	N D	T	3.6	8.1	10.1	12.9	14.8	13.9	4.8
A M	N D	T	0	0	1.4	7.9	13.2	14.0	9.1
P M	D	M	0.2	6.1	8.9	13.0	15.1	12.1	9.4
T W	D	M	3.9	7.8	8.0	9.9	10.5	8.8	6.0
			Mean 1.6	5.3	7.1	10.9	13.4	12.2	7.6
C—Orally—15 gm									
M G	N D	M	3.8	5.1	6.1	15.9	18.1	19.7	13.0
F G	N D	M	2.5	2.9	3.6	7.4	13.8	15.8	9.3
P D	D	T	1.0	5.2	8.4	14.5	12.3	13.2	4.4
M K	D	M	3.0	3.6	—	—	—	—	—
			Mean 2.6	4.2	6.0	12.6	14.7	16.2	8.9
D—Orally—20 gm									
S T	N D	T	—	—	22.4	—	—	21.4	16.7
J N	N D	M	4.4	8.4	9.2	21.3	15.6	15.2	13.8
A S	D	O	3.9	5.1	7.1	12.2	13.4	13.0	6.0
			Mean 4.2	6.8	13.4	16.9	14.5	16.5	12.8

\* Symbols D = diabetic N D = nondiabetic

† Symbols O = obese T = thin M = medium

‡ In 48 hours A M had a chlorpropamide level of 3.7 mg % and P M 5.4 mg %

### Serum Levels of Chlorpropamide After Continued Drug Administration

In view of the prolonged retention of the drug after a single dose, serum levels were studied in patients receiving continued daily doses. The amount prescribed was conservative in view of the previously mentioned experience in which 1 or 1.5 gm/day led to toxic symptoms. In general, 1 tablet of 500 gm was given as a single daily dose. After 1 or 2 weeks the dosage was cut to 250 mg/day in older and very thin patients. In some instances, the latter type of patients were given 250 mg/day from the outset. Since one

of the important purposes of this investigation was to ascertain possible cumulative effects after continued administration, frequent changes in dosage were avoided.

The above treatment was prescribed for 52 ambulatory diabetic patients who were given the medication from 6 to 26 weeks. Serial estimations of chlorpropamide concentrations were made of the blood, which was also analyzed for glucose content and hepatic and renal function profiles. The value of checking blood concentrations became apparent quite early. Four

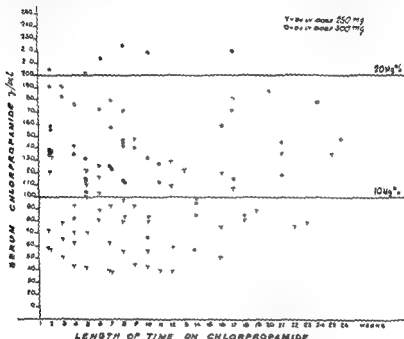


FIGURE 1 Serum chlorpropamide levels after prolonged administration

patients reported to the clinic regularly and insisted that they took the medication, yet zero levels of chlorpropamide were found in the blood. Two confessed later that they had not, but the other two individuals insisted to the end that they had taken the medication. Twenty-two patients took 500 mg/day, and 25 others took 250 mg/day.

In FIGURE 1 the blood levels of chlorpropamide are plotted against the length of time the drug was taken. In the patients taking 250 mg, most points fall between 4 and 10  $\mu\text{g}\%$  per cent. This range is lower than that found in a control group of normal patients given the same dose, as reported elsewhere in this monograph by Carlozzi and Iezzoni. The lower range might well be explained by patients' omission of occasional doses. There were 2 instances in which the blood levels were consistently elevated, it is



possible that these patients took aspirin, which interferes with the chemical estimation of chlorpropamide

With a daily dose of 500 mg the scatter was much greater. Again, 2 patients showed unexpectedly low values. A history of omission of doses could not be obtained in these patients, but the trustworthiness of such a history cannot be guaranteed. Serum chlorpropamide levels ranged from 11 to 22 mg per cent. In general the lower values were found in the obese patients and higher values in small, thin patients, but this could not be correlated statistically.

An observation constantly noted was that patients who had low serum drug levels on a given daily dose continued to have relatively low concentrations while receiving the same dose. The same finding occurred in patients who showed high serum levels in the first 2 or 4 weeks, they continued to have the higher concentrations for months if the dose was left unchanged. Thus, in the doses used, the blood level tended to be characteristic of the patient. After a plateau level was attained a cumulative effect was not noted.

#### *Relationship Between Drug Levels and the Hypoglycemic Effect After Single Doses*

Tests performed by estimating simultaneously the venous blood sugar levels and serum drug concentrations allowed the relationship between the 2 to be studied. The former declined at a slow continuous rate while the latter rose gradually. FIGURE 2 shows representative experiments in diabetics and nondiabetics. After 0.5 gm was administered orally, the blood sugar began to fall when the chlorpropamide concentration was between 2 and 3 mg per cent (FIGURE 2, T, C). The drop was maintained for 4 hours, when the drug levels were no higher than 6 mg per cent (FIGURE 2, L, F). The hypoglycemic potency of chlorpropamide at these low dosage levels posed the question as to what effect larger doses might have. When 2 g gm were given orally and the drug was slowly absorbed (FIGURE 2, A, S), the blood sugar also dropped slowly. When absorption was rapid, leading to initial high serum levels, the drop in blood sugar was rapid (FIGURE 2, J, T).

In practically all the oral experiments, the blood sugars continued to decline throughout the entire 4-hour period. This was found in both nondiabetics and diabetic responders. With the intravenous administration of the drug, however, nondiabetics showed a greater initial drop, after which the blood sugar tended to rise even when the drug blood levels were 10 and 12 mg per cent. In some cases this rise in blood sugar in the face of an adequate drug level was sufficiently marked to lead one to suspect that drug concentration in the liver or other organs might be more significant than that in the systemic blood.

#### *Antidiabetic Action of Chlorpropamide*

Many studies have reported an inability to correlate the single dose response of various oral hypoglycemic agents with their therapeutic efficacy. The blood sugar, although important is in many respects only an indirect

indicator of the complex and subtle alterations of metabolism leading to the well known complications that occur in a diabetic organism. Demonstration of the hypoglycemic activity of a drug does not necessarily denote that it is antidiabetic. Even exogenous insulin was under suspicion by the late H. Mosenthal who stated: "Insulin administered by injection, is not the complete remedy for diabetes, because the greater part of the injected insulin acts in the extrahepatic tissues and results in the deposit of glycogen in

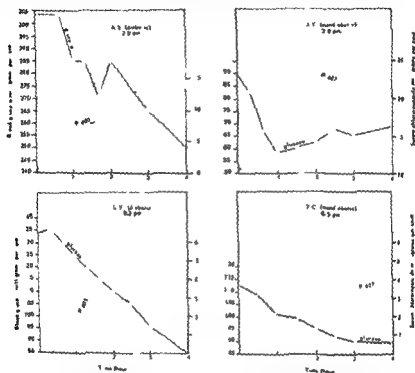


FIGURE 2 Blood sugar fall and chlorpropamide levels after a single dose of drug

them.<sup>1</sup> In this light a total evaluation of the antidiabetic properties of chlorpropamide must await many years of clinical observation. However, any attempt to initiate such an evaluation can begin by employing human diabetics of the maturity-onset type as test objects since such patients are known to respond to the drug.

Thirty-five of the 47 ambulatory patients who were selected for treatment with chlorpropamide were of this type and had previously been followed in the Diabetes Clinic for at least 6 months on other treatment. The aim of treatment in this clinic is to obtain a physiological level of control and optimal nutrition. The present group of patients was selected either because they

did not attain these criteria or because a comparison was desired between chlorpropamide and previous treatment. The patients kept charts of their urine tests (Clinetest) before meals and frequent fasting blood sugars were obtained.

The majority of our patients speak only Spanish, Italian or Arabic and live in families where the native dishes are chiefly of high carbohydrate content; obesity is common. In TABLE 3 (Group A) are assembled 15 uncomplicated obese mature diabetics of recent onset. According to past observations of Duncan<sup>8</sup> and of this clinic<sup>9</sup> probably all could be excellently controlled by weight reduction with a resultant increase in glucose tolerance. Varying degrees of lack of cooperation in dieting were the cause of poor diabetic control and all in Group A had not lost weight while presumably dieting. In the first 4 cases the addition of chlorpropamide was definitely of value obtaining a level of control that was not obtainable in the previous period. The fifth patient demonstrated that chlorpropamide does not allow unlimited eating of cake; she refused insulin therapy.

The next 7 patients had received insulin for at least 1 year prior to this study. Replacement of the insulin with chlorpropamide led to better, or at least equivalent results in all but 1 patient (N O C). Many of these patients volunteered the information that they previously had frequent mild attacks of hunger that had forced them to eat more and that they felt better without insulin. Despite these allegations weight loss was uncommon when on chlorpropamide.

The last 4 patients had previously received tolbutamide. With the exception of patient N O C who did badly with all attempts at treatment 0.25 gm. of chlorpropamide seemed to do as much as 1 gm. of tolbutamide. Control of these patients might have been better if larger doses of chlorpropamide had been used.

In TABLE 3 Group B is composed of a similar type of obese adults with diabetes of long standing. They had received insulin for 9 or more years and had remained obese. The replacement of the insulin with chlorpropamide led to better control in half these patients. Only 2 patients had previously been given tolbutamide; chlorpropamide in 0.5 gm. doses appeared to be slightly superior to 1.5 and 2 gm. of the former drug.

Diabetics who are thin pose a different problem. Adequate nutrition must be given and thus from the onset their diets contain a relatively large amount of carbohydrate. TABLE 4 shows the results with thin adult diabetics. Group C contains 3 patients who were always thin; developing diabetes later in life. The number was small but seemed to show the value of the oral

before coming to the clinic usually due to long-standing serious illness or previously undiagnosed diabetes. Insulin was usually prescribed when the patients first presented themselves to allow an ample diet to be given. When chlorpropamide was given a gradual reduction of

TABLE 3  
MADRID CITY ONSET TYPE OF DIABETES OBESE PATIENTS

Group A relatively recent onset no weight loss

Patient	Age	Duration (years)	Insulin (units) daily	Urine glucose (per cent)	Blood glucose (mg %)	Tolb* (gm) daily	Urine glucose (per cent)	Blood glucose (mg %)	P 607† (gm) daily	L-rine glucose (per cent)	Blood glucose (mg %)
PUG	51	1	0	0	186-238	—	—	—	0.5	0	116-140‡
POL	40	1	0	0	162-197	—	—	—	0.25	0	96-130‡
PIVZ	42	1	0	1.2	158-213	—	—	—	0.25	0	96-120‡
NYAD	48	6	0	0	200-266§	—	—	—	0.25	0	140-144
SAV	50	3	0	2	273-318§	—	—	—	0.5	0-1.4	225-259§
IOR	61	1	20 PZI	0-2	144-155	—	—	—	0.5	0-1.4	136-155
OLV	53	5	15 ∞ PH	1.2	140-197	—	—	—	0.25	0	96-140‡
OLV	64	1	20 ∞ PH	0	116-140‡	—	—	—	0.25	0	73-116‡
IVG	67	3	15 ∞ PH	0	160-202	—	—	—	0.25	0	102-138‡
WOL	71	2	25 ∞ PH	0	171-191	—	—	—	0.25	0	181-184
KOI	68	5	38 ∞ PH	0	181-202	—	—	—	0.25	0	163-176
NOC	65	4	35 ∞ PH	0-1.5	223-260§	3	0-1.4	181-298§	0.75	0.1	202-266§
ffV	46	6	0	0	244-250§	1.2	0	108-111‡	0.25	0	130-208
VIT	50	3	0	2	290-307§	1	0-3.5	156-176	0.25	0	162-181
VVJ	71	7	0	0-2	146-180	1	0-2	158-181	0.25	0	178-197

Group B Obese at onset long standing diabetes still of use

Patient	Age	Duration (years)	Insulin (units) daily	Urine glucose (per cent)	Blood glucose (mg %)	Tolb	Urine glucose (per cent)	Blood glucose (mg %)	P 607† (gm) daily	L-rine glucose (per cent)	Blood glucose (mg %)
KAR	59	11	0	0	190-240	1 (BZ SS)	0	160-161	0.25	0	113-140‡
QOI	67	11	35 ∞ PH	0-2	220-264§	2	0 tr	263-393§	0.5	0	176-197
QOI	67	16	25 ∞ PH	0-2	260-318§	1 S	1.4-3.4	360-396§	0.5	0	245-273§
LOV	56	11	35 ∞ PH	0	162-298§	—	—	—	0.5	0	232-310§
WPI	61	9	12 ∞ PH	0-2	197-232§	—	—	—	0.5	0-tr	113-148‡
PPI	57	21	20 PZI	0-2	218-219§	—	—	—	0.25	0	109-141‡

• **Tollutamide**

† Chloroquine made

Excellent control	urines always free of glucose	fasting blood sugar ■ to 120 mg per cent at most times
Good control	urines free of glucose	fasting blood sugar ■ to 120 mg per cent at most times
Fair control	urines free of glucose	fasting blood sugar ■ to 120 mg per cent at most times
Poor control	urines free of glucose	fasting blood sugar ■ to 120 mg per cent at most times
Very poor control	urines free of glucose	fasting blood sugar ■ to 120 mg per cent at most times

Had control urines usually contain sugar fasting blood sugar usually over 250 mg per cent

The maximum and minimum urine and blood sugars during the period of observation are given

TABLE 4  
MATURITY ONSET TYPE OF DIABETES THIN PATIENTS

Patient	Age	Duration (years)	Insulin (units) daily	Urine glucose (per cent)	Blood glucose (mg %)	Tolb* (gm) daily	Urine glucose (per cent)	Blood glucose (mg %)	P 1807† (gm) daily	Urine glucose (per cent)	Blood glucose (mg %)
Group C: always thin											
Q U I	65	8	—	0	166-200	—	—	—	0.25	0	150-197
M I R	84	2	—	0 tr	186-200	0.5	0	166-200	0.25	0	144-158
C A S	48	1	—	—	—	2	0	104-124†	0.25	0	120-112‡
Group D: thin obese at onset recent diabetes											
F A V	71	4	6.5 NPH	0	122-136‡	—	—	—	20 NPH 0.5	0-1‡	167-202
K I I	68	2	35 PZI	0.1	148-200§	—	—	—	0.5	0-tr	137-161
M I R	65	3	20 PZI	0	162-171	—	—	—	0.25	0	166-213
T O R	76	2	15 reg 1.5 NPH	0 tr	132-181	—	—	—	0.5	3-1	202-281§
Group I: thin obese at onset long standing diabetes											
J I S	64	14	24 NPH	0	96-180	—	—	—	0.5	0	124-163
S A D	73	18	25 PZI	0-1	213-334§	—	—	—	0.5	0	130-176
I I O	64	12	15 fente	1-2	213-218§	—	—	—	0.5	0	166-184
I R A	65	25	25 Globan	0-2	158-190	—	—	—	0.5	1-2	195-232§
S C H	71	21	25 NPH	0	197-208	6	0-2	220-258§	10 NPH 0.25	0	166-171
S U N	69	21	0	0	140-160	1.5	0	136-162	0.5	0	102-106‡
H O D	74	16	0	0-1‡	236-240§	—	—	—	0.25	0	225-232§

\* Tolbutamide

† Chlorpropamide

‡ Excellent control

§ Bad control

insulin was made in patients receiving 25 units or more. The insulin was abruptly stopped in those taking less.

It should be noted that the thin cases of recent onset (Group D) responded similarly to those of long standing diabetes (Group E). At the time that therapy with chlorpropamide was initiated, the level of control in these 11 patients (both groups) was excellent in only 1 patient and fair in only 2 even while on insulin. Subsequently on oral therapy after discontinuing insulin 5 patients (K, L, E, M, I, R, 7, I, S, S, A, D, and F, I, O) of the 11 demonstrated diabetic control that was equivalent to or better than when on insulin. Two cases who originally were badly controlled were markedly improved. One patient (S, C, H) who was completely unresponsive to 6 gm tolbutamide day obtained fair control with 10 U NPH and 0.25 gm chlorpropamide. The best result in this group was obtained in a cooperative patient (Y, U, N) who attained a physiological level of control and gained weight with 0.5 gm chlorpropamide but could not do so with diet alone or with 1.5 gm tolbutamide. This report represents a preliminary and therefore incomplete, study. Out of the total of 35 patients in TABLES 3 and 4 there were 7 primary failures. There is reason to believe that in many of our patients further manipulation of the dosage schedule might lead to better results.

It is important to reemphasize clinical results in treatment. The above charts giving urine and blood sugar level record only one of many criteria that should be used in assessing the antidiabetic quality of a medication. In our obese diabetics the mere prescribing of a diet without adequate cooperation by the patient had failed and many had such complaints as pruritis vulva and thirst alleviated by the oral hypoglycemic agent. In many thin adult patients chlorpropamide allowed better utilization of the ingested food leading to desired gains in weight. Such results can only be inferred from charts but again should be mentioned.

#### Toxicity

To study the incidence of toxicity the entire series of 47 ambulatory patients was reviewed. It has already been stated that with a daily dosage of 1.5 gm 4 out of 6 patients had severe asthenia, muscular weakness and lightheadedness. In the blood of one of these patients there was a drug level of 46.6 mg per cent and a blood sugar of 225 mg per cent after 2 weeks of medication. Such incidences of toxicity must be attributed to overdosage rather than to the drug itself. Recurrence of severe diabetic symptomatology occurred in 2 young adults with severe diabetes in whom the insulin was reduced when chlorpropamide was given. The latter should be attributed to nonresponsiveness rather than toxicity. Two patients without previous gastrointestinal complaints developed acute cholecystitis confirmed by surgery 1 and 2 months after taking chlorpropamide. These were probably coincidental.

In all 2 patients had pruritis while on therapeutic doses requiring discontinuance of the drug and 2 patients had dizziness and nausea. One of the patients with pruritis previously had a pruritic rash when taking tolbut

amide (Orinase) All symptoms quickly disappeared with discontinuance of the medication Serial hematological surveys on all patients have revealed no anemia a slight leukocytosis (10 to 11 thousand) with a normal differential leukocyte count was frequently encountered without adequate explanation Serial liver profiles also did not change In this connection it should be mentioned that 3 diabetic patients with known hepatic cirrhosis have been given chlorpropamide Liver biopsies had established the diagnosis in all but 2 had normal liver function tests and only 1 was active as shown by a markedly elevated serum transaminase The latter patient returned to normal while on chlorpropamide therapy The liver profile in the other 2 patients remained normal

The most serious manifestation of toxicity was encountered in a 56 year old man who previously had urticaria when given Orinase Immediately after the intravenous administration of 1 gm chlorpropamide, he went into a state of shock that responded to an intramuscular injection of Chlor Trimeton Blood sugars prior to and during the episode were 167 and 165 mg per cent respectively

### Summary

Chlorpropamide is an extremely potent hypoglycemic agent that is quickly absorbed and slowly excreted Oral administration in therapeutic doses leads to predictable drug levels in the serum It has demonstrated favorable antidiabetic activity during the period of study (6 months) and can effectively replace insulin in diabetics with maturity onset With it a better control can be obtained in that large segment of the diabetic population that does not fully cooperate in the dietary management Dosagewise it is more potent than Orinase Toxicity in therapeutic doses did not constitute a problem

### References

- 1 MOSENFELT, H O 1954 Control of the complications of diabetes hyperglycemia and glycosuria endogenous and exogenous insulin Am J Med Sci 227 134 140
- 2 DUNCAN G G 1935 Diabetes Mellitus and Obesity 7<sup>th</sup> Lea & Febiger Philadelphia Pa
- 3 HANDELSMAN M B & J K BRADFORD 1940 Treatment of diabetes mellitus in patients in the older age group N Y State J Med 40 1248 1259

# CLINICAL EXPERIENCE WITH CHLORPROPAMIDE\*

Barnett Greenhouse

Grace New Haven Community Hospital New Haven Conn

medication for diabetes

## Clinical Pilot Study

Chlorpropamide was subjected to a clinical trial in 50 nonhospitalized diabetic patients ranging in age from 18 to 87 with an onset age of 18 to 72 years and a duration of the disease from 1 day to 33 years. Two thirds of the patients studied have been on insulin for various periods to 33 years, and one third of these were on dosages of 50 to 150 U.

The patients were under close clinical observation and controlled by frequent laboratory tests designed to disclose any toxic effects on the liver, kidneys and blood forming organs. Laboratory profiles including blood sugar, urinalysis, peripheral hematogram, alkaline phosphatase, thymol turbidity and flocculation and blood urea nitrogen were done at weekly, biweekly and monthly intervals.

The compound was used in three basic clinical situations: (1) transfer from Orinase, (2) conversion from insulin, and (3) in newly discovered or previously untreated cases.

**Transfer from Orinase to chlorpropamide.** In the initial test series Orinase controlled diabetics (twenty two patients) were shifted to chlorpropamide for comparative evaluation on a gram for gram basis: an 0.5 gm tablet of chlorpropamide replacing an 0.5 gm Orinase tablet. Control of the diabetes proved adequate but chlorpropamide per day produced changes in one third of the patients. category of side effects of the hypoglycemic sulfonylureas with varying degrees of intensity. The subjective complaints were predominantly of a hypoglycemic nature with CNS circulatory and renal manifestations. Other side effects noted were gastrointestinal disturbances and allergic skin reactions.

There was no doubt that chlorpropamide lowered the blood sugar; the response was prompt and dramatic exhibiting a greater potency than Orinase.

The side effects were always reversible and gradually subsided when

Better tolerance was achieved  
 dosage although some patients

subsequently made available

\* The investigation reported here was supported by a research grant from Chas. Pfizer & Co. Inc. Brooklyn, N. Y.



with a lowering of the daily dose level below 500 mg, there was a decided improvement in clinical usefulness. Patients were successfully managed on the lower dosages with smooth control of hyperglycemia and glycosuria and with a low incidence of side reactions. Transfers from Orinase to chlorpropamide were subsequently made by replacing a 500-mg Orinase tablet with a 200 mg tablet of chlorpropamide. The medication was given in divided doses immediately after meals to minimize side reactions and to avoid gastric irritation.

Chlorpropamide was also found to restore control where Orinase effect declined or failed on long use (six patients). One insulin patient who did not respond to Orinase responded readily to chlorpropamide and discontinued insulin.

*Conversion from insulin to chlorpropamide* Generally where Orinase was known to be primarily ineffective chlorpropamide was also found to be

TABLE I  
DURATION OF CHLORPROPAMIDE THERAPY IN PATIENTS WITH DIABETES MELLITUS

Duration months	Number of cases			
	Total	Still on therapy	Lost to follow up	Therapy discontinued
1	8	—	—	8
2	8	3	—	5
3	10	8	—	2
4	2	2	—	—
5	5	2	—	3
6	1	1	—	—
7	7	5	—	2
8	8	8	—	1
Total	50	29		21

without effect in therapeutic dosages (six patients). Larger doses of chlorpropamide however tended to reduce the insulin dose in some resistant cases (four patients).

5

withdrawn  
added every  
day. At the same time the insulin was raised 2 to 4 U daily as needed to combat glycosuria.

## Greenhouse Clinical Experience

TABLE 2  
CLINICAL TOLERANCE FOR CHLORPROPAMIDE **Side Effects\***

Hypoglycemia (14)	
CNS (11)	agitation, blurred vision, confusion, dizziness, depression, dullness, drowsiness, fatigue, flushing, grogginess, headache, hunger, incoordination, irritability, lack of well being, lethargy, loss of concentration, malaise, nervousness, restlessness, sleeplessness, unsteadiness, weakness
Circulatory (2)	congestive failure, dyspnea, palpitation
Renal (1)	albuminuria, dyspnea, peripheral edema
Allergic Skin Reactions (4)	
Erythema Multiforme (1)	pruritis (2), urticaria (1)
Gastrointestinal disturbance (12)	
Anorexia, constipation, nausea, vomiting	diarrhea, distention, flatulence, halitosis, heartburn
Alcohol intolerance (1)	
Crystalluria ? (2)	
Sinking of red blood cells (1)	

\* Figure in parentheses gives number of cases

Actually the dosages of insulin and chlorpropamide were chosen arbitrarily and the patient was kept on the same dose of chlorpropamide as long as it proved effective and caused a decline in the insulin requirement. Meanwhile without anticipating the effect of the drug, a close watch was kept on the insulin need to keep the condition from getting out of control or to prevent insulin shock. The regimen lent itself readily to outpatient and office management and none of the patients required hospitalization.

*New and untreated diabetics.* Previously untreated and uncomplicated mild cases (4 patients) and a recently discovered adult diabetic patient responded promptly to chlorpropamide. As a routine a 200 mg tablet was given once or twice daily until the patient was sugar free then he was maintained on 1 tablet every morning after breakfast. Thereafter a second

### DISTRIBUTION OF PATIENTS AND RESULTS OF CHLORPROPAMIDE THERAPY IN 50 ADULT DIABETICS

#### RESULTS OF THERAPY

#### ORINASE TRANSFERS

#### CHLORPROPAMIDE & INSULIN

#### PRIMARY ORINASE FAILURES

#### SECONDARY ORINASE FAILURES

#### CONVERSION FROM INSULIN

#### NEW & UNTREATED DIABETICS



■ Adequate Control  
□ Inadequate Control

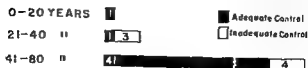
CORRELATION OF AGE AT TIME OF TREATMENT  
WITH RESPONSE TO CHLORPROPAMIDE

FIGURE 2

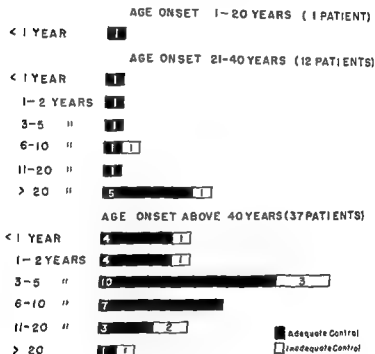
CORRELATION OF DURATION OF DIABETES WITH  
RESPONSE TO CHLORPROPAMIDE

FIGURE 3

tablet was allowed *after supper* whenever the dinner test was positive for sugar.

**Toxicity** Excluding toxic drug reactions inherent in overdosage, no persistent toxicity was encountered at therapeutic levels. About the only evidence of toxicity, which relates to other sulfonamides as well, has been the development of some skin rashes and itching (*four patients*). Otherwise, there was no evidence of toxicity that could be determined by studies of liver function, renal function, and the blood elements. No acetonuria was encountered in the responsive patients. *Dulling of the appetite was noted by some*

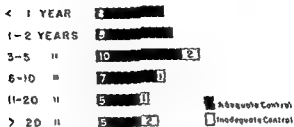
CORRELATION OF DURATION OF DIABETES WITH  
RESPONSE TO CHLORPROPAMIDE

FIGURE 4

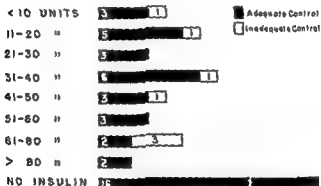
CORRELATION OF PRIOR INSULIN DOSE WITH  
RESPONSE TO CHLORPROPAMIDE

FIGURE 5

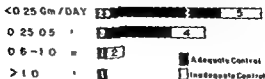
DAILY DOSAGE OF CHLORPROPAMIDE REQUIRED  
FOR MAINTENANCE CONTROL

FIGURE 6

# EXPERIENCE WITH CHLORPROPAMIDE THERAPY DISCONTINUED

NO OF PATIENTS 50  
UNDESIRABLE SIDE EFFECTS 6  
SUGAR-FREE 9  
INEFFECTIVE 6

FIGURE 7

**Clinical Results** One half of the group of 50 patients studied has been under continuous treatment for 3 to 8 months (TABLE 1) approximately one fourth of the patients were eliminated in the first 2 months because of side effects (TABLE 2) or lack of response to chlorpropamide. Adequate control was obtained in 44 patients of whom 8 were maintained on chlorpropamide and insulin; results were inadequate in 6 (FIGURE 1).

Chlorpropamide was found to be effective chiefly in patients more than

who have had diabetes for as long as 25 years (FIGURE 4). Patients on prior

tween 100 and 400 mg (FIGURE 6).

The drug was stopped in nine patients who became sugar free; it was ineffective in six patients; and six patients abandoned medication because

TABLE 3  
CORRELATION OF DAILY DOSAGES OF CHLORPROPAMIDE WITH NUMBER OF  
SIDE EFFECTS

Side effect	Grams per day			
	<0.25	0.5	1.0	>1.0
Hypoglycemia and sequelae			14	
Gastrointestinal	1		11	
Skin		1	2	1
Alcohol intolerance		1		
Red blood cell stippling	1			
Crystalluria (?)	2			

Glycosuria and elevated blood sugars recurred after two to six months in three patients who had been on chlorpropamide therapy one of these was previously shifted to chlorpropamide because of secondary failure on Orinase

### Comment

Preliminary and early use of chlorpropamide was undertaken without previous knowledge of the clinical effects of the drug During the exploratory phase guided by experience with Orinase chlorpropamide was first administered dose for dose to a selected group of Orinase controlled patients for comparative evaluation The response was prompt and unquestionable Chlorpropamide proved to be a potent and rapidly active hypoglycemic agent with an activity more than twice that of tolbutamide Doses in the magnitude of 10 gm were found to induce adverse side effects that for the most part were correlated with hypoglycemic manifestations and sequelae characteristic of the long acting insulins Expanding trials however showed chlorpropamide to be generally well tolerated and clinically effective in the therapeutic range of 100 to 400 mg and to have no significant undesirable by effects

In view of its enhanced potency and rapid action as observed clinically treatment with chlorpropamide was initiated without a priming dose In the newly treated diabetic the response to the drug was found to be rapid with a minimum dose from the start for conversion from insulin a basic dose

or relatively mild diabetics of the middle age or older group respond best to the drug The responsiveness of the early case is very striking The mild diabetics and by that token the older patients are very sensitive to the drug

significant  
propam

doses tolerated beyond the therapeutic range and it may thus prove to be a truer measure of drug responsiveness than Orinase

A significant observation involves the effectiveness of chlorpropamide in primary and secondary Orinase failure The development of a refractory tolerance to Orinase experienced on prolonged use of the drug is becoming evident also with chlorpropamide Stopping medication at intervals to allow for a rest period and starting again when and if glycosuria recurs are measures aimed at preventing drug resistance

Chlorpropamide and Orinase have been used interchangeably and, currently a shift from one to the other also is being explored as a possible means of avoiding an acquired tolerance to either

Hypoglycemic reactions on small doses (200 mg) of chlorpropamide have been observed in sensitive individuals indicating the need for a maintenance dose to the lowest level consistent with good control

The management of diabetes with chlorpropamide is

of the marked blood sugar fluctuations and wide metabolic excursions characteristic of insulin. Patients feel better and are less agitated, and excessive appetite is curbed by the anorectic effect of the drug, as well as by the normalization of the blood sugar. Absence of acetonuria in the chlorpropamide-responsive patients reflects a favorable effect on carbohydrate metabolism with a stabilizing influence on the diabetic state. See case reports below.

### *Summary*

Chlorpropamide (P 607 Diabinese), a new sulfonylurea derivative with a hypoglycemic activity similar to that of Ornase, but with enhanced potency and longer duration of action, is currently under clinical investigation.

An early pilot study and expanding trials with this compound give promise of its therapeutic value in the treatment of diabetes. It is proving to be an active and clinically effective oral hypoglycemic agent more than twice as active as tolbutamide. In the therapeutic range of 100 to 400 mg, it appears to be safe, effective, and well tolerated, with minimal side reactions.

### *Acknowledgment*

I thank D. G. Iezzoni of the Clinical Research Department, Chas. Pfizer Co., Inc., Brooklyn, N. Y., for his interest and aid in the project reported in this article.

CASE REPORT 1  
 Primary Orinase 1 ulcer

# ORAL DIABETIC THERAPY - DIABETES Conversion and Progress Report

Date	Urine Sugar Test				Urine Ketone Test				NPH Insulin				Dosage	
	A M	P M	P M	P M	A M	P M	P M	P M	A M	P M	P M	P M		
1958														
Apr 22	0	0	0	0	BS*	150				20				500 mg
23	0	0	0	0						18				500
24	3	0	0	0						22				500
25	0	0	0	0						18				500
26	0	0	0	0						16				500
27	1	1	1	1						16				500
28	1	0	0	0						20				500
29	0	0	0	0						18				500
30	0	0	0	0						16				500
May 1	0	0	0	0						14				500
2	0	0	0	0						12				500
3	0	0	1	1						10				500
4	0	0	1	1						8				500
5	3	3	1	1						12				500
6	0	0	0	0						12				500
7	0	0	0	0						10				500
8	0	0	3	3						8				500
9	3	0	0	0						10				500
10	0	0	0	0						8				500
11	0	0	0	0						6				500
12	0	0	3	3						4				500
13	0	0	0	0						4				500
14	1	0	0	0	BS	138				6				500
Insulin discontinued on May 20, 1958														

C N female age 48 weight 150 A diabetic on insulin for 22 years and currently on 30 U of NPH insulin and 10 gm of Orinase She was started on 2.0 mg Dialinase and 70 U NPH insulin daily and promptly became sugar free In 1 week she had an insulin reaction on one-half the previous insulin dose and gradually discontinued insulin

\* All blood sugars were performed at random by the Folin Wu method capillary blood sugar method and expressed in milligrams per 100 cc of blood



CASE REPORT 2  
Secondary Failure on Orinase and Diabinese

ORAL DIABETIC THERAPY - DIABINESE  
Conversion and Progress Report

Date	Urine Sugar Test				Urine Keto Test				NPH Insulin				Doseage Per Day
	AM	PM	PM	PM	AM	PM	PM	PM	AM	PM	PM	PM	
	AM	PM	PM	PM	AM	PM	PM	PM	AM	PM	PM	PM	
1958													
Apr 21	4	0	0	0	BS*	226			80				250 mg
22	4	1	0	0					80				750
23	4	0	0	0					80				750
24	1	0	0	0					80				750
25	4	0	0	0					80				750
26	4	0	0	0					80				750
27	0	2	0	0					80				750
28	0	0	0	0	BS	143			80				750
29	0	0	0	0					80				250
30	0	0	0	0					60				250
May 1	tr	0	0	0					50				250
2	0	tr	0	0					45				250
3	tr	0	0	0					40				250
4	0	4	4	4					30				250
5	0	0	0	0					30				250
6	1	0	0	0	BS	217			25				250
7	0	0	0	0					20				250
8	0	0	0	0					15				250
9	0	0	0	0					10				250
10	0	0	0	0					0				250
11	0	0	0	0					0				250

R M male age 46 weight 257 A diabetic for 4 years, he was originally converted to Orinase from 97 U of NPH insulin. He continued sugar free on 10 gm Orinase daily for 7 months when his diabetes decompensated and he went back on insulin to 125 U daily. His response to Diabinese was rapid and insulin was discontinued again. Glycosuria recurred after 2 months and he was shifted to insulin and Orinase.

\* All blood sugars were performed at random by the Tolin Malmros capillary blood sugar method, and expressed in milligrams per 100 cc. of blood.

Case Report 3  
Insulin ConversionORAL DIABETIC THERAPY - DIABINESE  
Conversion and Progress Report

Date	Urine Sugar Test										Urine Keto Test				NPH Insulin				Doseage		
	AM					PM					AM	PM	AM	PM	AM	PM					
	1	2	3	4	5	1	2	3	4	5	1	2	1	2	1	2					
1958																					
May 30	0	0	0	0	0	0	0	0	0	0	BS*	216			20	18			200 mg		
May 31	0	0	0	0	0	0	0	0	0	0					16	14			200		
June 1	0	0	0	0	0	0	0	0	0	0					12	10			200		
2	0	0	0	0	0	0	0	0	0	0					8	7			200		
3	0	0	0	0	0	0	0	0	0	0					6	5			200		
4	0	0	0	0	0	0	0	0	0	0					5	6			200		
5	0	0	0	0	0	0	0	0	0	0					5	5			200		
6	0	0	0	0	0	0	0	0	0	0					5	5			200		
7	0	0	0	0	0	0	0	0	0	0					5	5			200		
8	0	0	0	0	0	0	0	0	0	0					5	5			200		
9	0	0	0	0	0	0	0	0	0	0					5	5			200		
10	0	0	0	0	0	0	0	0	0	0	BS	158			0	0			200		
11	0	0	0	0	0	0	0	0	0	0											
	Insulin discontinued																				

W W male age 68 weight 174 A diabetic for 14 years patient was taking 20 to 30 U of NPH insulin. He was put on 20 units of NPH with 200 mg of Diabinese daily. He continued sugar free gradually reducing his insulin and discontinuing it in less than 2 weeks.

\* All blood sugars were performed at random by the John Malmros capillary blood sugar method and expressed in milligrams per 100 cc of blood

## CASE REPORT 2

Secondary Failure on Orinase and Dialinase

# ORAL DIABETIC THERAPY - DIABINESE Conversion and Progress Report

ORAL DIABETIC THERAPY - DIABINESE														Patient Name R M					
Conversion and Progress Report														Chart No 3538					
Date	Urine Sugar Test						Urine Keto Test						NPH Insulin				Dosage per Day		
	AM	AM	PM	PM	PM	PM	AM	AM	PM	PM	PM	PM	AM	AM	PM	PM			
1958																			
Apr 21		4		0			BS*	226							80			250 mg	
22		4		1											80			750	
23		4		0											80			750	
24		1		0											80			750	
25		4		0											80			750	
26		4		0											80			750	
27		0		2											80			750	
28		0		0			BS	143							80			250	
29		0		0											60			250	
30		0		0											50			250	
May 1		tr		0											45			250	
2		0		tr											40			250	
3		tr		0											30			250	
4		0		4											30			250	
5		0		-											25			250	
6		1		0			BS	217							20			250	
7		0		0											15			250	
8		0		0											10			250	
9		0		0											0			250	
10		0		0											0			250	
11		0		0											0			250	

\* All blood sugars were performed at random by the John Valmor's capillary blood sugar method, and expressed in milligrams per 100 cc of blood

R M male age 46 weight 257 A diabetic for 4 years he was originally converted to Orinase from 97 U of NPH insulin. He continued sugar free on 10 gm Orinase daily for 7 months when his diabetes decompensated and he went back on insulin to 125 U daily. His response to Diabinese was rapid and insulin was discontinued again. Glycosuria recurred after 2 months and he was shifted to insulin and Orinase.



Case Report 4  
Insulin Conversion and Withdrawal of Diabinese

ORAL DIABETIC THERAPY - DIABINESE  
Conversion and Progress Report

Date	Urine Sugar Test						Urine Keto Test						Insulin		Dosage Per Day
	AM	AM	PM	PM	PM	PM	AM	AM	PM	PM	PM	PM	AM	PM	
1958															
May 3															
4	4		2				BS*				144				500 mg
5	0		2												500
6	0		tr												500
7	0		0												250
8	0		0												250
9	0		0												250
10	0		0				BS				97				200
11	0		0												200
12	0		tr												200
13	0		tr												200
14	0		0												200
15	0		0												200
16	0		0												200
17	0		0												200
18	0		0												200
19	0		tr												-
20	0						BS				135				100
	Diabinese Discontinued														0

\* All blood sugars were performed at random by the John Malmros capillary blood sugar method, and expressed in milligrams per 100 cc of blood



# EXPERIENCES WITH TOLBUTAMIDE AND CHLORPROPAMIDE IN TUBERCULOUS DIABETIC PATIENTS

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Although the literature regarding the action, use, and results of treatment of diabetes with tolbutamide (Orinase) is voluminous and detailed, there has been very little recorded about the use of this drug in the treatment of diabetes coexistent with active pulmonary tuberculosis. Inasmuch as these two diseases usually complicate each other, it seemed appropriate to study the effects of this oral antidiabetic drug in such cases. When the discovery of chlorpropamide 1 propyl 3-(*p* chlorobenzenesulfonyl)urea was announced a comparative study of the two drugs seemed to be a logical and desirable project.

## *Tuberculous Diabetic Patients Treatment with Tolbutamide, House Diet, and Intituberculosis Chemotherapy*

A group of 9 patients with far advanced active pulmonary tuberculosis and coexistent diabetes mellitus were treated with tolbutamide and standard house diet (about 3000 calories per day). Although all patients in this group had active tuberculosis, none had any signs of toxicity such as fever, chills, emaciation or hemorrhage. Tuberculosis treatment in all cases consisted of daily isoniazid (INH) and *p* aminosalicylic acid (PAS).

Seven patients were males, two were females. Their ages ranged from 42 to 83 years.

**Diabetic histories.** In three patients the diabetes had been present for several years. One of these had been well controlled with 40 to 50 U of NPH insulin (case 1). The other two cases (2 and 4) had been controlled

1. Diet without insulin

1.

n

16

fourth (case 7) required 75 U

In two patients the diabetes was discovered during hospitalization for tuberculosis. One was found 21 days after admission (case 3) and the other, five months after admission (case 5).

These two patients had diabetes, with only one requiring 75 U. None of these patients had ever had an episode of acidosis. As these patients were not ideal candidates for tolbutamide, they were

included in the study to determine if any benefit might be derived from oral antidiabetic therapy.

Let us now see how the patients fared before the start of therapy, after one week of the tolbutamide treatment, and after one month of the tolbutamide treatment.

(WBC) and fasting blood sugar (FBS). The sugar was checked by 4 urine sugar tests daily and by frequent FBS studies.

The duration of tolbutamide therapy in all cases was too brief (22 days to 4 months) to determine any secondary failures. Insulin was omitted the 1st before starting the diet. A basal dose of 2 gm was given for 3 days. When the blood sugar was not satisfactorily controlled, the dose was increased to 4 gm daily.

TABLE 1 represents a tabulated summary of this study. The penultimate column indicates that only one patient (case 3) was satisfactorily controlled by tolbutamide. In this very small series this represents a 12 per

TABLE 1  
TUBERCULOUS DIABETICS ON FREE DIET AND TOLBUTAMIDE

	Patient	Sex	Age	Activity of tuberculosis	Diabetic history	Type of diabetes	Insulin units	Insulin control	Tolbutamide control	Duration on tolbutamide
1	D M	♂	63	Inact	4 yr	Mod	40-50	Good	Poor	22 days
2	B F	♀	75	Mod	9 yr	Mod	35-50	Good	Poor	3 mo
3	H C	♀	59	Act	1 mo	Mod	30	Good	Good	4 mo
4	J B	♂	83	Mod	1 yr	Mod	20-40	Good	Poor	3 mo
5	E M	♀	54	Act	2 mo	Mod	30-45	Good	Poor	3 mo
6	K N	♀	76	Mod	20 mo	Mod	40-50	Poor	Poor	1½ mo
7	J R	♂	57	Mod	9 mo	Mod	75	Good	Poor	1½ mo
8	J S	♂	42	Act	8 mo	Mod	15	Good	Poor	2 mo
9	C R	♂	70	Act	2 yr	Mod	55	Poor	Poor	2 mo

cent rate of success. Therefore we conclude that diabetic patients who require a free high calorie diet for the treatment of their tuberculosis even if the diabetes is only of moderate degree are not good prospects for successful tolbutamide control. In such cases insulin remains the drug of choice.

In all the cases studied laboratory studies remained normal (with the exception of urine sugar and FBS in the failures) and no toxic side effects were observed.

#### *Tuberculous Diabetic Patients on Restricted 2000 Calorie Diet Treatment with Tolbutamide and Antituberculosis Drugs*

Ten tuberculous diabetic patients were given a 2000 calorie diet containing fat 80 gm protein 100 gm and carbohydrate 200 gm (TABLE 2). Five of the patients in this group were also members of the first group (unrestricted diet). The same preliminary studies were made and the same tolbutamide dosage was given.

Before starting tolbutamide the single-dose test was made in all ten patients.

Except for case 7 who showed no response 4 hours following the ingestion of 3 gm of Orinase the results of the single-dose test varied from 18 to 52 per cent reduction of blood sugar with an average of 29 per cent.

There was no correlation between the effect of the single-dose test and the



remaining six patients, who required more than 30 U of insulin, did not respond well

In the chlorpropamide group, 8 of the 9 patients had disease that was controlled with 30 U or less of insulin. These six patients showed a good response to chlorpropamide, and were well controlled. The remaining three cases had previously required more than 30 U for adequate control, and these patients did not do well on chlorpropamide.

From these observations, we conclude that in our small groups of tuberculous diabetic patients the efficacy of both drugs is dependent on the mild or moderate nature of the disease. Those patients requiring less than 30 U of insulin for control do well with either drug. This conclusion is further supported by the fact that, of the seven patients who were studied in both drug groups, the same patients did well with both drugs and the remaining patients did badly with both drugs.

Although our studies were limited to a small number of patients, we feel that we can state as a clinical guide that a tuberculous diabetic patient, even with far advanced pulmonary disease who is not toxic and who is on a 2000-calorie diet will do well with either tolbutamide or chlorpropamide if his insulin requirement for control is 30 U or less. On the other hand, patients requiring even a little more than 30 U failed to respond to either drug.

#### *The Action of Isoniazid on Carbohydrate Metabolism*

Isoniazid (isonicotinic acid hydrazide) has been for the past several years one of the drugs commonly used in the treatment of tuberculosis. There has been only rare mention of the effect of INH on carbohydrate metabolism and on the fasting blood sugar; these reports have been primarily in the foreign literature. We believe that our preliminary observations (TABLE 4) in this area might be of more than academic interest and that this subject merits further investigation.

TABLE 4  
HYPOGLYCEMIA EFFECT OF ISOINIAZID

Patient	Sex	FBS mg per cent	BS 4 hr after 500 mg INH		FBS mg per cent	BS 4 hr after tolbutamide 3 gm		FBS mg per cent	BS 4 hr after tolbutamide and INH	
			mg per cent	% de- crease		mg per cent	% de- crease		mg per cent	% de- crease
1 J. B.	♂	327	285	13	344	293	24	259	171	34
2 A. B.	♂	191	182	5	217	130	40	222	126	43
3 K. X.	♂	327	283	13	318	159	19	370	309	17
4 C. A.	♀	450	413	0	490	490	0	467	467	0
5 H. C.	♀	207	146	26	176	130	26	163	101	35
6 E. M.	♀	234	154	31	271	154	43	278	118	57
7 A. C.	♀	420	359	15	443	345	21	348	285	18
8 J. D.	♂	182	201	10*	212	126	41	150	104	30

In a group of 8 tuberculous diabetic patients, the following procedures were performed (1) tolbutamide single dose test using 3 gm of the drug, (2) a similar test using 500 mg of INH (with determination of the FBS before, and 4 hours after, ingestion of 500 mg of INH) and (3) a similar test using both 3 gm of tolbutamide and 500 mg INH given simultaneously.

**Results.** The tolbutamide single dose tests produced blood sugar reductions ranging from 19 to 43 per cent, with an average decrease of 28 per cent of the initial FBS level. One patient showed no response.

In the INH single-dose test one patient showed an increase in the FBS of 10 per cent. The patient who showed no response to tolbutamide showed no response to INH. The remaining 6 patients showed a significant reduction of the blood sugar, ranging from 5 to 34 per cent with an average reduction of 18 per cent.

When the combined single-dose test (3 gm Ormase and 500 mg of INH) was given to the same group, there were reductions in the blood sugar ranging from 17 to 57 per cent with an average of 35 per cent.

From these studies it is apparent that INH has a definite effect on carbohydrate metabolism; in almost every case INH exerts a hypoglycemic effect that is not an insignificant one and when combined with tolbutamide, seems to enhance the action of this drug.

These observations suggest an area of investigation that hitherto has not been reported in the search for oral control of diabetes.

#### Summary

The efficacy of two oral antidiabetic agents in cases of diabetes with coexistent pulmonary tuberculosis (active but not toxic) was studied and compared. These two agents are tolbutamide and chlorpropamide. The salient observations resulting from this study are as follows:

The results of treatment with tolbutamide and chlorpropamide in tuberculous diabetics are essentially the same.

Tuberculous diabetic patients who require free diet ('house diet') because of their tuberculosis are not good candidates for treatment with either of the oral antidiabetic agents. This is true even if the diabetes is mild.

Tuberculous diabetic patients on a 2000-calorie diet show good response to both tolbutamide and chlorpropamide in cases of mild diabetes that were previously controlled by 30 U of insulin or less. It is interesting to note that if insulin requirements were even slightly more than 30 U, neither drug was effective in maintaining adequate control.

The extent of tuberculosis did not seem to influence the activity of either drug. All patients studied have active disease but were not toxic.

Preliminary studies of the effects of isoniazid, the most commonly used drug today in the chemotherapy of tuberculosis on the blood sugar, revealed a significant hypoglycemic effect in almost every case. When compared with one of the oral antidiabetic drugs (tolbutamide), the hypoglycemic effect was greater than with either INH or the antidiabetic drug alone. In other words, isoniazid enhanced the hypoglycemic effect of the antidiabetic drug. This suggests further possibilities for the oral drug control of diabetes.

greatest fall in blood sugar levels occurred at the 60 min interval and continued to 120 min. The mean percentage fall at 60 min was 30 per cent, with a range of 3 to 55 per cent. At 20 min the mean percentage fall was 11 per cent, with a range from a 10 per cent rise to a 30 per cent fall. At 40 min, the mean percentage fall was 23 per cent with a range from a 1 per cent rise to a 44 per cent fall. It is to be noted that, at this time interval,

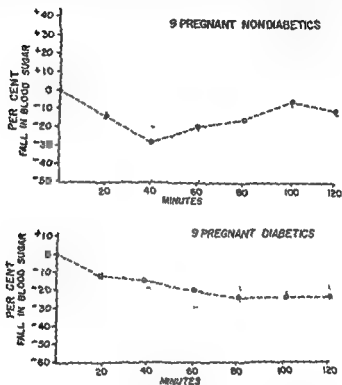


FIGURE 2 The mean tolbutamide tolerance curves of nine pregnant nondiabetics and nine pregnant diabetics. Individual responses are recorded.

the blood sugar levels of 3 patients (11 per cent of this group) were able to fall below the greatest mean percentage fall of the nondiabetic group. Thus

of the diabetic group is characterized by a maximum at 60 min or more, and demonstrating no significant hypoglycemic responsiveness.

The response to intravenous tolbutamide is not altered in pregnancy (FIGURE 2 upper graph). In a group of 9 nondiabetic pregnant patients, the greatest mean percentage fall was 28 per cent, and occurred at 40 min. The range of variation was 4 to 52 per cent. They also demonstrated a considerable degree of hypoglycemic responsiveness. In the group of 9 preg

nant diabetic patients, the greatest mean percentage fall occurred at 80 min, with a range of 10 to 35 per cent. Within 120 min there was no hypoglycemic responsiveness of any significance. Of these 9 patients, only 1 whose glucose tolerance was abnormal demonstrated a normal tolbutamide response.

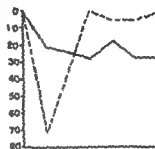
### CASE I GLUCOSE TOLERANCE TESTS

	7 MONTHS	POSTPARTUM
F	86	115
1/2 HR.	180	105
1 HR.	172	133
2 HR.	142	112
3 HR.	150	102

#### TOLBUTAMIDE CURVE

———— INTRAPARTUM

----- POSTPARTUM



### CASE II GLUCOSE TOLERANCE TESTS

	7 MONTHS	POSTPARTUM
F	81	77
1/2 HR.	131	132
1 HR.	144	131
2 HR.	148	86
3 HR.	93	74

#### TOLBUTAMIDE CURVE

———— INTRAPARTUM

----- POSTPARTUM

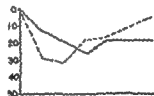


FIGURE 1 Curves of two patients whose mildly abnormal glucose and tolbutamide tolerance at seven months pregnancy was reversed completely in the postpartum period.

In 2 instances, both glucose tolerance and tolbutamide curves were studied in the intra and postpartum periods (FIGURE 1). The upper part of the chart demonstrates a mildly abnormal glucose tolerance curve characterized by a blood sugar of 142 mg per cent at 2 hours. The tolbutamide curve is also considered to be abnormal, demonstrating both a delayed fall and no significant return. The postpartum glucose tolerance test shows a reversion toward normal. The tolbutamide curve shows a completely normal response characterized by a rapid fall and a rapid return. The lower section of the chart shows a similar situation where both abnormal glucose tolerance and tolbutamide curves at 7 months reverted to normal in the postpartum period.

### Discussion

It is only in instances of suspicious mild diabetes, either in or out of pregnancy, that it is difficult to arrive at a definite diagnosis. If

circumstances additional measures beyond the glucose tolerance test have been wanting. The response of the blood sugar in a 2 hour period to an intravenous injection of sodium tolbutamide separates the mild diabetic from the normal patient if 2 conditions are fulfilled: first the fall in blood sugar should be prompt and should reach its lowest level by 40 min. and second

promptly with a brisk fall in blood sugar levels the remainders of their curves are also in a downward direction. In a very few instances was there a minimal irregular upward turn. Analysis of the latter part of many more curves it is hoped will allow the degree of hypoglycemic responsiveness to be expressed mathematically. We should like to emphasize the fact that Moyer's glucose tolerance criteria clearly separated the normal from the abnormal when correlating the tolerance curves with the tolbutamide curves. There were no patients with blood sugars higher than 140 mg. per cent at 2 hours who had a normal tolbutamide response. Nine patients with distinctly abnormal tolbutamide curves had 2 hour blood sugars between 140 mg. per cent and 160 mg. per cent.

The procedure is not associated with any significant side effects. Only two patients had early in the test hypoglycemic symptoms that required the administration of glucose.

We feel that further experience with this procedure will permit greater accuracy in detecting the mild diabetic state.

### Summary

The mild diabetic response demonstrates a lesser fall with no significant hypoglycemic responsiveness.

- (3) Pregnancy does not alter the tolbutamide response in normal patients.
- (4) It is felt that this procedure will help in detecting the mild diabetic state.

### Acknowledgment

We express our thanks to Martin G. Goldner of the Jewish Chronic Disease Hospital Brooklyn N. Y. for his suggestions.

### References

1. BRAVERMAN A. C. & W. DREY & S. SHERRY. 1956. Experience with Orinase in the management of adult diabetes. *Metabolism* 5(2) 911.
2. UNGER R. H. & L. L. MADISON. 1958. Comparison of response to intravenously administered sodium tolbutamide in mild diabetic and non diabetic subjects. *J. Clin. Invest.* 37 62.
3. MOYER J. H. & C. R. WOMACK. 1950. Glucose tolerance: a comparison of four types of diagnostic tests in 103 controlled subjects and 26 patients with diabetes. *Am. J. Med. Sci.* 219 161.
4. FOLIN O. & H. W. 1918. *J. Biol. Chem.* 41 367.

# THE INTRAVENOUS TOLBUTAMIDE RESPONSE TEST IN THE DIAGNOSIS OF MILD DIABETES

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The striking difference in response to intravenously administered sodium tolbutamide exhibited by nondiabetics and mild diabetics has been reported in detail elsewhere.<sup>1,2</sup> More recently the clinical usefulness of a standardized

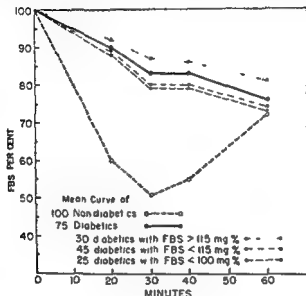


FIGURE 1. Mean tolbutamide response curves of all but five of the subjects studied.

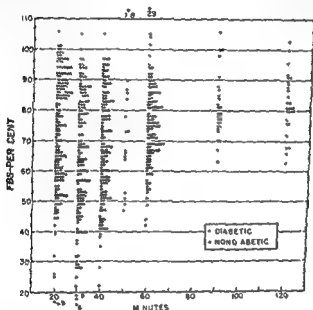
of the *Journal of Clinical Investigation*, Volume 37, 1958

intravenous tolbutamide response test in the diagnosis of mild diabetes mellitus has been delineated and diagnostic standards suggested.<sup>3</sup> This paper therefore will merely review briefly certain aspects of these earlier reports as they pertain to the experiences of Zarowitz and Eis<sup>4</sup> with this test.

Zarowitz and Eis have provided further valuable evidence to support the qualifications of the intravenous tolbutamide response test as a diagnostic procedure for mild diabetes. In addition they have extended experience with the test to the pregnant state. The correlation between reversible

abnormal tolbutamide response curves and reversible abnormal glucose tolerance curves in the intra- and postpartum periods, which they have observed, is consonant with the hypothesis that both tests measure the same physiological function

Clinical usefulness of a diagnostic test for mild diabetes mellitus depends on its ability to differentiate the diabetic without fasting hyperglycemia from the nondiabetic. That the intravenous tolbutamide test qualifies in this respect is indicated by the data in FIGURE 1. It will be noted that the response curve of the mildest diabetics with fasting normoglycemia closely

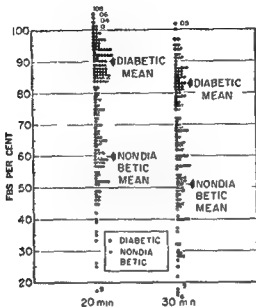


parallels the gently sloping curve of the more severe diabetics and is in distinct contrast to the rapid fall in blood glucose level typical of the non diabetic group. This fall reaches a nadir between 20 and 40 min and is generally followed by a vigorous rebound to or toward the pretest value. Zarowitz and Eis report similar results.

Zarowitz and Eis have emphasized the diagnostic value of the rebounding phenomenon, which, as they point out, seldom occurs except test beyond the 40 min point. While there is no doubt as regards the nondiabetic group, we have regarded this phenomenon as being secondary to the more rapid decline in blood glucose level exhibited by the group, that is, the result of counterregulatory forces thus set into play. Rebound, in our opinion

reflects both the rate and extent of decline in blood glucose level and the  
 rate of decline rather than beta cell func

be expected to mirror most directly beta cell responsiveness to this stimulus



In FIGURE 2 in which the tolbutamide response curves of 100 nondiabetics and 75 mild diabetics are recorded maximum separation of the 2 groups occurs at 20 and 30 min. A close up of these points is provided in FIGURE 3. At 20 min 95 per cent of the diabetic group have a blood glucose level of 80 per cent or more of the pretest value whereas 96 per cent of the nondiabetic group have a level of less than 84 per cent of that value. Selection of 84 per cent and more as the diabetic zone and 80 per cent and less as the nondiabetic zone has provided standards that have thus far been 95 per cent accurate. The zone between 80 and 84 per cent of the pretest level is considered presumptively abnormal but nondiagnostic.\*

\* These standards apply only if blood glucose is determined by the Somogyi-Nelson technique. It is hoped that the data of Zarowitz and his will provide diagnostic standards for the Folin-Wu method.



The 30-min specimen (FIGURE 3) appears to be diagnostically more specific inasmuch as 99 per cent of the nondiabetics are at less than 77 per cent of the pretest level by this time. However, this specimen is far too insensitive to be of value in the exclusion of diabetes, since 15 per cent of mild diabetics have reached the nondiabetic zone at this point.

It would seem that the blood glucose response within the first 30 min of the tolbutamide injection provides the necessary diagnostic information in the vast majority of individuals. Termination of the test at this point

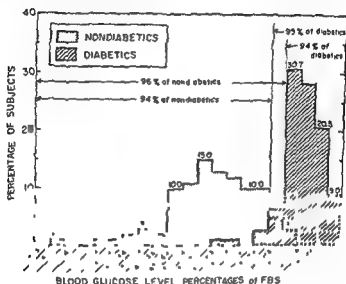


FIGURE 4 The frequency distribution of the blood glucose levels (in percentages of FBS) 20 min after tolbutamide injection.

endows it with an asset unique among diagnostic tests for mild diabetes, namely, brevity. Furthermore, it shortens the period of hypoglycemia that occurs in the nondiabetic group. If, however, the data of Zarowitz and Eis should indicate that examination of the rebound phase of the curve provides an unmistakable increase in diagnostic accuracy, prolongation of the test may be necessary.

As in the case of the glucose tolerance test<sup>14</sup> it would be naive to expect that the tolbutamide response test can provide a sharp demarcation between

zone of overlap includes 10 per cent of each group and extends from 75 to 89 per cent of pretest level. Within this zone rigid adherence to the recommended diagnostic criteria can lead to error since only probability of disease can be estimated. For example, these limited data suggest that among persons whose levels fall to between 75 and 79 per cent of the pretest value the diagnostic probability of diabetes is 21 per cent; between 80 and 84 per cent of pretest level it is 56 per cent; and between 85 and 89 per cent it is 91 per cent. Above and below the overlap zone diagnostic accuracy approaches 100 per cent.

It is our tentative impression that the zone of overlap of the tolbutamide response test is somewhat smaller than that of the oral glucose tolerance test due primarily to fewer false positive results. It would be premature, however, to pass final judgment on the standards proposed for the tolbutamide response test in view of the controversy that still prevails in respect to the oral glucose tolerance test despite several decades of extensive use.

At present, however, the intravenous tolbutamide response test appears to qualify as an accurate, safe, rapid, and not unpleasant diagnostic procedure for mild diabetes that compares favorably with other methods now commonly used for this purpose.

### References

1. UNGER R. H. & L. L. MADISON: 1957. A new diagnostic test for mild diabetes mellitus. *Clin. Research Proc.* 6: 187.
2. UNGER R. H. & L. L. MADISON: 1959. Comparison of response to intravenously administered sodium tolbutamide in mild diabetic and non-diabetic subjects. *J. Clin. Invest.* 37(4): 627.
3. UNGER R. H. & L. L. MADISON: 1958. A new diagnostic test for mild diabetes mellitus: evaluation of the intravenous tolbutamide response test. *Diabetes* 7: 445.
4. ZAROWITZ H. & B. E. H. 1949. The role of a tolbutamide tolerance test in the detection of the mild diabetic state: a preliminary report. *Ann. N. Y. Acad. Sci.* 74(3): 612.
5. UNGER R. H.: 1957. The standard two-hour oral glucose tolerance test in the diagnosis of diabetes mellitus in subjects without fasting hyperglycemia. *Ann. Internal Med.* 47(6): 1138.
6. UNGER R. H. & L. L. MADISON: 1959. Evaluation of the diagnostic and prognostic specificity of the standard oral glucose tolerance test by means of long term follow up studies. Proc. 4th Congr. Intern. Diabetes Federation, in press, Dusseldorf, Germany.

## A CLINICAL COMPARISON OF CHLORPROPAMIDE AND TOLBUTAMIDE\*

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Chlorpropamide, a recent orally administered hypoglycemic agent, has been compared to tolbutamide with regard to acute and chronic hypoglycemic potency and clinical effectiveness. The chemistry and animal studies of these two sulfonylureas are discussed elsewhere in this monograph.

### *Material*

Studies were conducted on 26 subjects including 1 normal and 25 diabetic. Five of the diabetics were hospitalized and maintained throughout their course on a constant diet of known composition with a daily intake of carbohydrate ranging from 150 to 220 gm. and protein from 80 to 120 gm. Both acute and chronic potency of the drugs were studied under these conditions. The normal subject was allowed a free diet between comparative studies of the 3.0 gm. dose. Sufficient time was allowed between the acute dosages of each drug to prevent interference with the subsequent hypoglycemic response. One of the diabetics had a previous total bilateral adrenalectomy and another had diabetes secondary to a total pancreatectomy. Sixteen of the diabetics were studied for 5 months as outpatients in the diabetes clinic of the University of California School of Medicine.

### *Methods*

Blood sugars were measured by the method of Nelson and Somogyi.<sup>1</sup> Urinary glucose was measured by the glucose oxidase method.<sup>2</sup> Serum chlorpropamide levels were determined by a procedure developed by the Chemical Research and Development Department of Chas. Pfizer & Co., Inc., Brooklyn, N. Y.<sup>3</sup> The content of unesterified fatty acids in the blood was obtained by the Davis extraction procedure,<sup>4</sup> followed by titration according to the method of Dole.<sup>5</sup>

### *Results*

*Acute hypoglycemic effects.* Various amounts of chlorpropamide and tolbutamide were administered as a single dose to both normal and diabetic subjects in order to compare the acute hypoglycemic effectiveness of each. Serum values of chlorpropamide and blood sugar levels were measured.

\* The study reported in this paper was aided by a grant from Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

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York, N. Y.

simultaneously to study the acute relationship between the hypoglycemic effect and the concentration of the drug in the blood and to observe the degree and rapidity of absorption. In FIGURE 1 the effect of the 0.5 gm dose on 3 diabetic patients is noted. The blood sugars are measured in the fasting state for the first 4 hours. It is apparent that the onset of hypoglycemia is within the first 2 hours with both drugs and continues for 4 hours

# 0.5-GM PO SINGLE DOSE

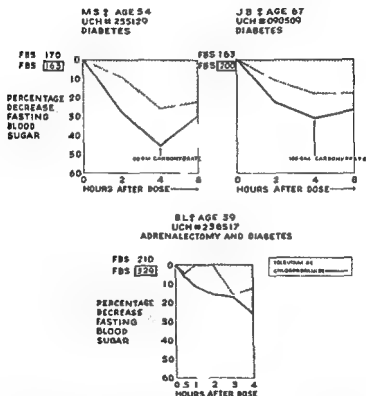


FIGURE 1. Comparative acute hypoglycemic potency of chlorpropamide and tolbutamide following 0.5 gm. as single oral dose.

before the standard meal is given. Postprandially there is a rise toward the euglycemic level. The comparative hypoglycemic potencies of chlorpropamide is greater in each case than that of tolbutamide by a factor approaching 2 when the areas representing blood sugar fall are compared. In the lower part of FIGURE 1 the effect of a similar dose given orally to an adrenalectomized patient maintained on cortisone replacement alone shows a minimal response to tolbutamide becoming significant 3 hours after the dose is given.

## 10-GM P.O. SINGLE DOSE

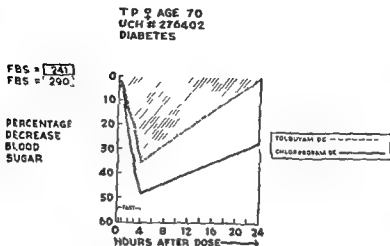


FIGURE 2 Comparative acute hypoglycemic potency of chlorpropamide and tolbutamide following 10 gm as single oral dose

## 30-GM P.O. SINGLE DOSE

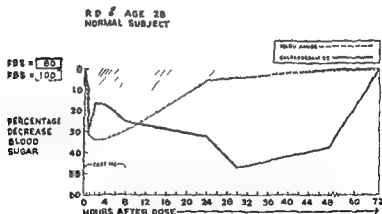


FIGURE 3 Comparative acute hypoglycemic potency of chlorpropamide and tolbutamide following 30 gm as single oral dose

Chlorpropamide, however, causes a much more significant fall starting within 30 min after oral administration. Here the acute hypoglycemic potency of chlorpropamide is greater than that of tolbutamide by a factor greater than 2.

FIGURE 2 illustrates the comparative effect of the 10-gm dose of the

2 compounds. Again it may be noted that chlorpropamide has a hypoglycemic potency approaching 2 times that of tolbutamide when the areas of hypoglycemia for similar time intervals are measured.

In FIGURE 3 the 3 gm dose of each compound administered to a normal subject demonstrates that at this dose level the comparative hypoglycemic potency of chlorpropamide is greater than 2 times that of tolbutamide when

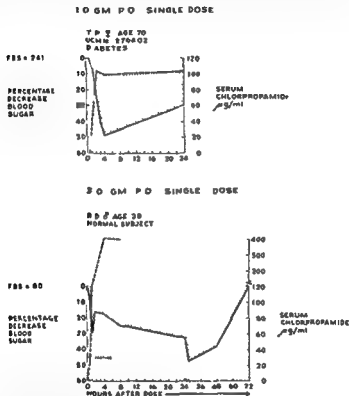


FIGURE 4 Serum levels of chlorpropamide compared to changes in blood sugar at the 10 gm dose (upper) and 30 gm dose (lower)

the total areas of blood sugar fall below the base line are compared. How

in blood sugar below base line levels that lasts less than 48 hours

It would appear that the intrinsic potency of each drug has reached a maximum at this dose level and that their hypoglycemic effects are equal, acutely. Their over all hypoglycemic potency, however, varies with the differences in duration of activity.

*Blood levels of chlorpropamide and its hypoglycemic effectiveness* - The serum levels of chlorpropamide measured simultaneously with blood sugar levels (shown in FIGURE 4) follow the 10 gm and 30 gm single oral dose. It may be noted that the rate of increase and levels achieved for the serum values of chlorpropamide are proportional to the dosage. These suggest that absorption is rapid.

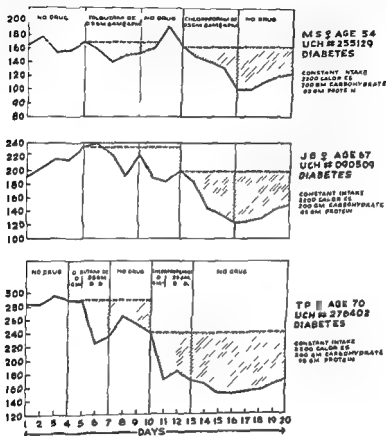


FIGURE 5 Comparative chronic hypoglycemic potency of chlorpropamide and tolbutamide compared at similar dose levels and time intervals

... that are also sug  
 25 per cent  
 achieve this  
 dose, and  
 s are lower  
 between 20 and 100 µg  
 have been obtained plasma values for tolbutamide in 2 previous studies

demonstrated by the fact that, as compared to tolbutamide, a lower serum value is required to achieve a significant hypoglycemic effect.

**Chronic hypoglycemic effects** The comparative hypoglycemic potency upon repeated daily administration was studied in 3 hospitalized diabetic patients maintained on the metabolic ward on constant dietary intake for prolonged periods. FIGURE 5 demonstrates that the relative potency of chlorpropamide is again greater than that of tolbutamide in all of the individuals studied. Upon interruption of intake of each drug the hypoglycemic effect of the former persists longer, and fasting blood sugars remain below their original levels for 4 days. In patient T P 7 days after stopping the drug the fasting blood sugar levels had not returned to their previous base line levels. Twenty four hour urine sugars measured in these studies were generally below 100 mg for 24 hours and were not changed significantly during therapy. The comparative areas of hypoglycemia reveal that the chronic potency of chlorpropamide upon repeated daily administration is of the order of 6 times that of tolbutamide.

**Rate of disappearance of chlorpropamide** The apparent discrepancy between acute and chronic potency of chlorpropamide is most probably related to the prolonged rate of disappearance of this compound in the blood. Half life periods were calculated based on the serum level of chlorpropamide following cessation of medication. TABLE 1 shows that the half life rate of disappearance of chlorpropamide in 3 patients at different dose levels ranges from 36 to 39 hours. In 2 previous studies tolbutamide revealed a half life ranging from 4 to 6 hours as calculated from the disappearance of the compound from the plasma.<sup>1,2</sup> These figures are close to the sixfold increase in chronic potency for chlorpropamide compared to tolbutamide previously shown. In TABLE 1 we note that the serum level of chlorpropamide following repeated daily administration at doses of 0.5 gm daily and 200 mg 3 times daily remains steady during continued administration. It is apparent that the serum levels of chlorpropamide have risen to more than the effective levels previously determined to be between 20 and 120  $\mu\text{g/ml}$ . In addition the levels reach a plateau with repeated daily administration and remain relatively constant thereafter. Thus after 72 and 107 days of treatment respectively in the two cases serum levels remain between 250 and 350  $\mu\text{g/ml}$  throughout. Fasting blood sugar levels are not related to serum levels of chlorpropamide once the steady levels have been reached although good control of hyperglycemia has been achieved in each case.

**Mechanisms of action** The possible mechanism of action of this new sulfonylurea compound appears to be similar to those of tolbutamide and the other sulfonylureas previously studied. Thus, in FIGURE 6 we note that the fasting blood sugar levels are not significantly affected in a depancreatized diabetic who received first saline and then chlorpropamide intravenously. This is in agreement with previous studies on other sulfonylurea compounds in animals<sup>3</sup> and in man.<sup>4</sup> Repeated daily administrations of chlorpropamide



TABLE 1  
RELATIONSHIP OF ORAL DOSAGE TO SERUM LEVELS OF CHLORPROPAMIDE AND TO BLOOD SUGAR LEVELS

## Drug elimination

Case	Subject	Dosage	Duration of therapy	Time after stopping dose (hours)	Serum levels of chlorpropamide $\mu\text{g/ml}$	Calculated rate of disappearance of chlorpropamide (half life)	Fasting blood sugar
1	B K Normal	1.0 gm	single dose	0 0.5 1 2 4 6 8 24	0 0 31 91 114 164 137 102	38 hours	80 87 55 67 104 96 68 82
2	R D Normal	3.0 gm	single dose	0 4 8 24 32 48 72	0 400 400 420 305 180 112	39 hours	80 68 57 54 43 50 92
3	J B Diabetes	0.5 gm twice daily	start day 1 start day 2 start day 3 start day 4	16 60 ■	0 67 116 187 177 69 48	36 hours	200 182 155 133 125 130 150

## Chronic drug levels

4	L S Diabetes	500 mg daily	start day 1 start day 11 start day 23 start day 45 start day 74 start day 107		0 350 250 320 260 252		200 138 88 116 130 99
5	P P Diabetes	200 mg thrice daily	start day 1 start day 4 start day 32 start day 51 start day 72		0 260 320 250 310		242 133 94 81 125

administration. The appearance of these  
are not a direct effect of the sulfonylurea

— that of  
glycemic  
diabetic

agent alone. In some cases, however, the  
acidosis, with wide variations in daily levels of fasting blood sugars and in

their daily dosage of insulin. All 3 were found to be primary failures on chlorpropamide therapy at doses ranging from 0.5 gm to 1.5 gm daily and demonstrated steadily increasing blood sugar levels and ketosis within the first 3 to 4 days of therapy. It is apparent therefore that functional beta cells are required for the action of chlorpropamide just as they are for the other sulfonylureas.

Finally, chlorpropamide was given in repeated daily doses to an adrenalectomized patient maintained on cortisone alone. In FIGURE 7 a rather exaggerated hypoglycemic response may be observed. The 24 hour urinary

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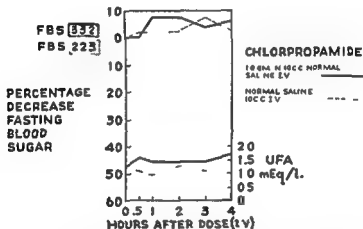


FIGURE 6. Acute effects of chlorpropamide (and a similar volume of saline given intravenously) on the fasting blood sugar and unsaturated fatty acid levels of a depancreatized diabetic.

sugars shown in the lower part of the figure parallel the fasting blood sugars in onset and duration of fall. This suggests that chlorpropamide may have a depressant action on the gluconeogenesis induced by steroid administration, an important factor in this patient's hyperglycemia.

**Outpatient trials.** A limited number of stable diabetics was given chlorpropamide in the diabetes clinic. Criteria for selection of patients were the same as those used to select candidates for tolbutamide therapy. None had

had been treated on dietary regimen alone without success. Three patients had been well controlled on small doses of insulin and 8 patients had been on tolbutamide previously with good control and no untoward effects. It

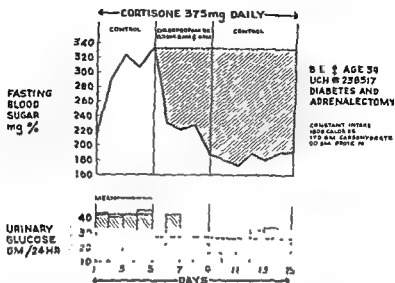


FIGURE 7 Increased sensitivity and prolonged hypoglycemic effect of chlorpropamide administration to an adrenalectomized diabetic patient

was found that 7 patients complained of nausea soon after starting chlorpropamide. Doses in excess of 600 mg daily were consistently associated with nausea, reducing the dosage below this level was generally followed by relief of symptoms. Four patients however, had to be returned to their

TABLE 2  
OUTPATIENT TRIALS ON CHLORPROPAMIDE

Total number of patients	16
Unstable diabetics (all primary failures)	3
Stable diabetics	13
Previous dietary treatment alone	2
Previous insulin	3
—	8
—	7
—	4
—	9
—	2
—	2
Previous corticosteroid	5
Dosage tolbutamide	1 to 2 gm daily
Control	good
Untoward symptoms	none
Duration of treatment	6 months to 2 years
Dosage, chlorpropamide	250 to 600 mg daily
Control	good
Untoward symptoms	none
Duration of treatment	3 months to 8 months

from 3 to 6 months of treatment. Repeated hematologic, hepatic, and renal studies have revealed no toxic effects of the drug to date.

In our clinical experience the maintenance dose of chlorpropamide was found to be one third to one quarter that of tolbutamide. However considering the chronic potency and the difference in the rate of disappearance from the blood a relative dosage ratio of 1.5 or 1:1 should prove effective. Further clinical studies may show this to be so.

### Discussion

The present study suggests that chlorpropamide is a significantly more potent hypoglycemic agent than tolbutamide both in acute and chronic activity. The former appears to be determined merely by the intrinsic

half life of the drug in body fluids in its active form. This was found to be 36 hours instead of the 6 hours for tolbutamide, and might thus account for the difference in chronic potency between the 2. It was interesting to note that the serum level of chlorpropamide reached a relatively steady plateau not exceeding 350  $\mu\text{g}$  per ml following prolonged periods of administration at the dosage of 0.5 gm daily.

As to the mechanism of action, chlorpropamide would appear to require the presence of functioning beta cells of the pancreas just as do the other sulfonylureas previously studied. Chlorpropamide did not effect a decrease in blood

ones showing that higher dosages of sulfonylureas than those necessary to produce hypoglycemia may inhibit insulin degradation.

A slightly exaggerated hypoglycemic effect observed in an adrenalectomized patient on cortisone therapy suggests that chlorpropamide may also be an inhibitor of action on gluconeogenesis.

The maintenance dosage of chlorpropamide appears to lie between one third and one sixth that of tolbutamide. From our experience we conclude that if this is kept at less than 500 mg per day, the incidence of nausea is markedly reduced.

### Summary

Chlorpropamide has approximately twice the hypoglycemic activity of tolbutamide in its acute effect for a four hour period.

ac  
s/r  
to be six times greater than that of tolbutamide.

The mode of action of the two sulfonylurea derivatives appears to be identical.

The prolonged action and tendency to nausea, when 500 mg daily is exceeded suggest that care must be observed in its clinical use.

### Acknowledgments

We acknowledge the cooperation of Herbert C. Moffitt, Jr., chief of the diabetes clinic of the University of California Department of Medicine, Robert Reiss, and other members of the diabetes clinic. We are indebted to Richard Havel for the studies mentioned under *Methods*.

## References

- 1 NELSON N 1944 A photometric adaptation of the Somogyi method for the determination of glucose J Biol Chem 163 375-380
- 2 FROESCH E R & A E RENOLD 1956 Specific enzymatic determination of glucose  
D L B K I G
- 3            "            "
- 4            proteins Arch Biochem 16 351-358
- 5 DOLE V G 1956 A relation between non-esterified fatty acids in plasma and the metabolism of glucose J Clin Invest 36 150-154
- 6 STOWERS J M R P MAHLER & R B HUNTER 1958 Pharmacology and mode of action of the sulfonylureas in man Lancet i 278-283
- 7 MORTIMORE G E V C DiRAIMONDO & P H FORSHAM 1956 Metabolic effects of Orinase in diabetes including two cases complicated by other endocrinopathies Metabolism 5 840-846
- 8 HOUSSEY B A J C PENHOS N TEODOSIO J BOWKETT & J APFELBAUM 1957 Action of the hypoglycemic sulfonyl compounds in hypophysectomized adrenalectomized and depancreatized animals Ann N Y Acad Sci 71 (1) 12-24
- 9 OGRYZLO M A & J HARRISON 1956        "        "        "        "        "  
creatic diabetes following pancreatectomy
- 10 BIERMAN E L V P DOLE & T N R  
esterified fatty acid metabolism in diabet
- 11 DOLE N P 1958 Fat metabolism in d i a
- 12 MIRSKY A I G PERISUTTI & D DIENGOI  
hypoglycemic sulfonamides Metabolism 5 156-161

# EXPERIENCES WITH CHLORPROPAMIDE WITH REFERENCE TO ITS INTERMITTENT USE

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Our experiences with chlorpropamide were obtained in the Middle East where there are two reasons for a tremendous need for an oral antidiabetic preparation. First the standards of hygiene and the habits of living make the use of insulin difficult if not hazardous. Second the people are accustomed to a gigantic consumption of carbohydrate. Arabic bread is indeed the staff of life and carbohydrate is consumed at the rate of 500 to 800 gm./day. If the production of other foodstuffs were feasible from an economic and cultural point of view many diabetics might be controlled by weight reduction and a shift in the source of calories. This is not now possible hence an oral antidiabetic agent is most valuable.

TABLE I  
BLOOD LEVELS OF CHLORPROPAMIDE\* IN MG PER CENT ON A DAILY ORAL INTAKE  
OF 1 CM AND CESSATION ON 8TH DAY

Patient No	Days											
	1	2	3	4	5	6	7	8	9	10	11	12
13	5.8	5.9	12.2	9.2	22	26	18	15.6	9	10	5	4
14					20	21	15	10	4.5	5.5	2.5	1.5
17	6.3	11	12	14	16	14	13	11	12	6.8	—	2.5

\* Sulfylelly Max (Gahwyler of Chas Pfizer & Co. Inc. Brooklyn N. Y.)

Chlorpropamide has certain characteristics that apart from its hypoglycemic qualities make it of special use in the management of diabetes. Upon the daily oral administration of a fixed amount the blood level rises gradually to a maximum in 5 to 7 days. If the administration is then interrupted the blood level falls very slowly so that there may be a concentration of 4 to 5 mg. per cent even after 5 days. This is illustrated in the figures furnished by Gahwyler on blood levels (TABLE I). This suggested the possibility that the drug could be given intermittently.

Our patients were admitted to the hospital where diet and activity could be controlled. The patients selected the diet they thought they could maintain for the period of study. They were followed up on an out-

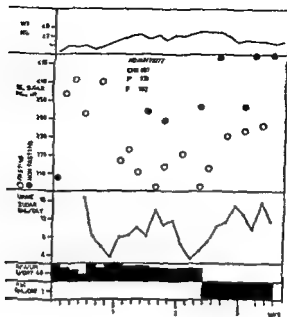


FIGURE 1 Unstable juvenile diabetic.

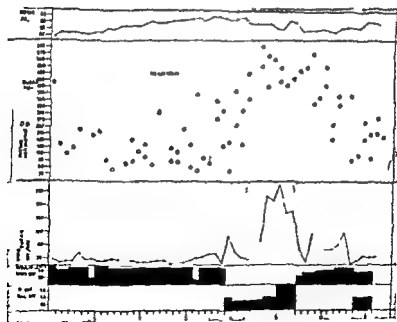


FIGURE 2 Severe diabetic with renal glycosuria

Our detailed studies with chlorpropamide are illustrated by the following cases

The first patient was an 18 year old girl (N D 99277) with diabetes of 7 months duration chlorpropamide did not control her diabetes in any way (FIGURE 1)

The second patient was a 50 year old farmer (M S 126347) with diabetes of 4 years duration and a low renal threshold for sugar His diabetes was severe and again could not be controlled by chlorpropamide (FIGURE 2)

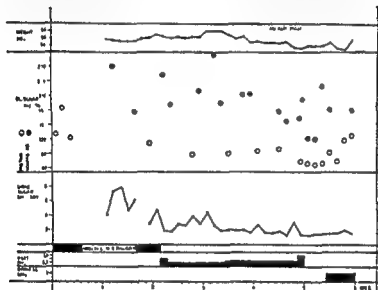


FIGURE 3 Mild diabetic

It was interesting to note that there was no apparent effect on his renal glycosuria. The concomitant use of chlorpropamide with insulin did not appear to reduce the insulin dosage substantially.

The third patient was a 60 year old, a former wrestler (M K 125037) with mild diabetes for four years (FIGURE 3). He had been taking 20 units of insulin irregularly. He had proteinuria, slight edema and a slightly low serum albumin of 2.9 gm per cent. After a period of stabilization on insulin he was changed to chlorpropamide. On a daily maintenance dose there was no evidence of hypoglycemia, but the interesting feature was that a single dose of 1.5 gm maintained his blood sugar and 24 hour urine excretion within normal limits for 72 hours and without hypoglycemia (FIGURE 4). There was no evidence of acceleration of the diabetic vascular damage while on chlorpropamide. His eyegrounds, proteinuria, and hypoalbuminemia remained constant. His urea nitrogen fluctuated between 15 and 27 mg per cent.

The fourth patient was a 50 year old man (K D 89351) with diabetes of



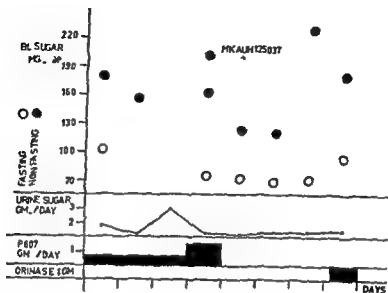


FIGURE 4 Mild diabetic with prolonged response to chlorpropamide

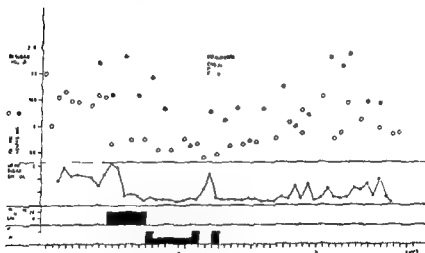


FIGURE 5 Mild diabetic with prolonged response to chlorpropamide

very small doses of chlorpropamide kept him controlled his blood and urine sugar

The fifth patient was a 60 year-old woman with diabetes of four months

duration. Difficulties in her control by intermittent chlorpropamide are shown in FIGURE 3.

The studies have been extended to include our clinic patients whose diet and activity cannot be controlled. In five patients taking 0.5 gm of chlorpropamide every two to three days, the nonfasting blood sugar could frequently be maintained at the upper limits of normal. However, this was much less striking than in the third patient (M. K. 125037), whose 72 hour control probably represented the delayed excretion of a cumulative dose. On

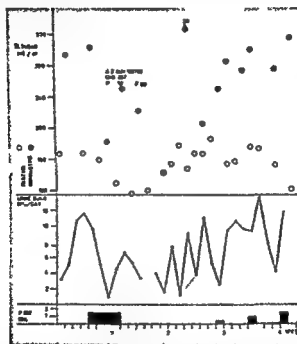


FIGURE 6 Mild diabetic with inadequate response to intermittent chlorpropamide

the other hand, our fourth patient (K. M. 89351) maintained normal values for a very long time after a relatively small dose of chlorpropamide. The fifth patient represents some of the difficulties inherent in attempting intermittent dosage.

We have not had the opportunity to study the specific effects of this drug on the complications of diabetes. In the cases studied, there was no evidence for any acceleration of the degenerative processes in retinal, coronary, or renal circulations.

The intriguing aspect of chlorpropamide is its mechanism of action, particularly, how this drug can, while circulating at slowly decreasing blood levels, maintain the blood sugar within normal limits despite three ingestions of carbohydrate a day.

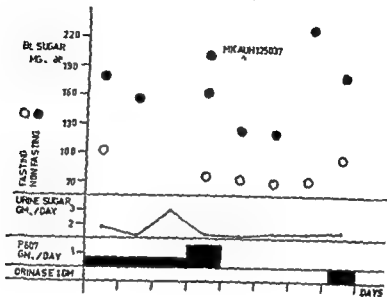


FIGURE 4. Mild diabetic with prolonged response to chlorpropamide

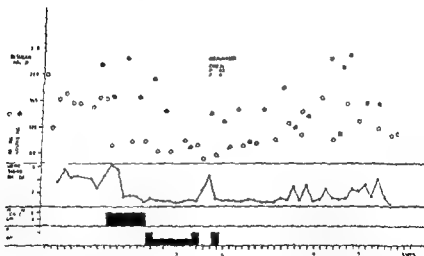


FIGURE 5. Mild diabetic with prolonged response to chlorpropamide

2 years' duration. He was a mild diabetic and of a comfortable, middle-aged habitude that resists all changes in weight (FIGURE 5). On his particular diet the need for 20 units of insulin is demonstrated, and it is apparent that very small doses of chlorpropamide given at considerable intervals controlled his blood and urine sugar.

The fifth patient was a 60-year-old woman with diabetes of four months'

duration. Difficulties in her control by intermittent chlorpropamide are shown in FIGURE 6.

The studies have been extended to include our clinic patients whose diet and activity cannot be controlled. In five patients taking 25 gm of chlorpropamide every two to three days, the nonfasting blood sugar could frequently be maintained at the upper limits of normal. However this was much less striking than in the third patient (M. K. 125037) whose 72 hour control probably represented the delayed excretion of a cumulative dose. On

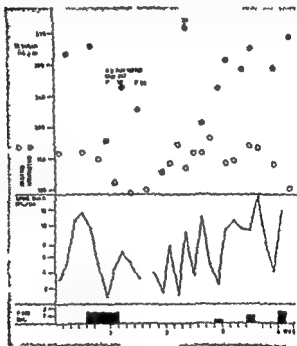


FIGURE 6 Mild diabetes with inadequate response to intermittent chlorpropamide.

#### Intermittent Dosage

We have not had the opportunity to study the specific effects of this drug on the complications of diabetes. In the cases studied there was no evidence for any acceleration of the degenerative processes in retinal, coronary or renal circulations.

The intriguing aspect of chlorpropamide is its mechanism of action, particularly how this drug can while circulating at slowly decreasing blood level maintain the blood sugar within normal limits despite three ingestions of carbohydrate a day.

*Conclusion*

In occasional patients the slow excretion of chlorpropamide may permit its intermittent use

*Discussion of the Paper*

J D N NABARRO (*Middlesex Hospital London England*) From the purely clinical standpoint the question that must be answered is Does chlorpropamide have any advantages over tolbutamide? Chlorpropamide is certainly easier for the patient to take and it can be given once daily whereas tolbutamide must be given in divided doses

A major problem with tolbutamide at the present time is the development of acquired or secondary resistance to its hypoglycemic action This is not the same as secondary failure a term used by several contributors to this monograph Secondary failure is more often due to the fact that the patient has broken his diet than to acquired resistance to the hypoglycemic drug and it is not reasonable to blame the drug for the indiscretions of the patient Acquired resistance can be established only if the patient is hospitalized and remains hyperglycemic while on a strict diet or if a high dosage of tolbutamide (3 gm /day) can be withdrawn without any further rise of blood sugar Our incidence of acquired resistance is 10 per cent for patients on the drug for more than 6 months and it has developed between the seventh and seventeenth months of treatment One patient with acquired resistance was transferred to chlorpropamide without adequate response

We have had to withdraw tolbutamide in 7 per cent of our patients because of side effects 4 per cent abdominal and 3 per cent skin changes One patient who was unable to take 1.5 gm of tolbutamide because of abdominal discomfort is now well controlled without symptoms on 0.25 gm of chlorpropamide daily

One final point that I should like to make is that we must not underestimate the length of time required for proper assessment of the value of these drugs We have recently examined all our data on tolbutamide treated patients and have found that the percentage still on the drug and in satisfactory control drops steadily over the first 18 months However between 18 months and 3 years the figure levels out at about 35 per cent of those giving a good initial response This of course tells us nothing about the antidiabetic value of the drug and it relates only to its hypoglycemic action

# A CLINICAL AND PHARMACOLOGICAL COMPARISON OF CHLORPROPAMIDE AND OTHER SULFONYLUREAS\*

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The effective blood level of chlorpropamide† probably lies between 4 and 16 mg per 100 ml which is comparable with those of carbutamide and tolbutamide but chlorpropamide differs significantly from tolbutamide in that it is not readily metabolized to a physiologically inactive compound as tolbutamide is to carboxy tolbutamide<sup>1</sup>. In fact the ultraviolet absorption spectrum of chlorpropamide in the blood seems to be identical with that of the drug in the urine suggesting that it has undergone no chemical change

TABLE 1  
MEAN HALF LIFE AND PERCENTAGE OF BODY WEIGHT IN WHICH THREE SULFONYLUREAS WERE DISTRIBUTED

	Half life hours	Volume of distribution as percentage of body weight
Carbutamide	40 (8)	approx. 45
Tolbutamide	3.5 (6)	approx. 20
Chlorpropamide	34.5 (8)	approx. 18

Numbers of individuals in parentheses

This effective blood level of chlorpropamide can be maintained with as little as 0.5 gm. day in contrast to the three- or fourfold dosage that is required for tolbutamide. Both drugs are rapidly absorbed; they produce peak serum levels 2 to 3 hours after ingestion. The volume of distribution of both drugs in the body is similar (TABLE 1) and approximates the volume of the extracellular fluid whereas carbutamide is distributed in about twice as large a volume. The half life of chlorpropamide that is the time required for any given serum level to fall by 50 per cent. is approximately 10 times longer than that of tolbutamide and nearly as long as that of carbutamide (TABLE 1). FIGURE 1 illustrates how these data were obtained. A single dose for example 2 gm. of a sulfonylurea is given to an individual who has not previously received this drug. Blood samples are then withdrawn at intervals determined by the anticipated half life and the concentrations of the drug are estimated in these samples using a controlled serum taken before the experi-

ment started. When these concentrations are plotted on a log scale against time a straight line is obtained. This line can then be extrapolated back to zero time, and the theoretical initial concentration is then read from the scale. The assumption is made that all the drug administered was absorbed and that no significant fraction was excreted before absorption was complete. Having thus derived the theoretical initial concentration, the time at which

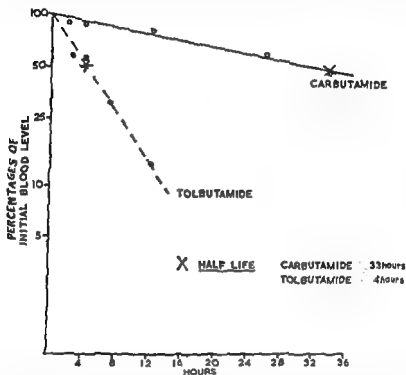


FIGURE 1. Rate of fall in serum level of carbutamide and tolbutamide after a single administration of 3 gm. of each to the same individual at different times. The theoretical initial concentration is obtained by extrapolating the line representing the rate of decrease of blood levels recorded on a log percentage scale. Reproduced by permission of *The Lancet*.

If half-life fallen by 50 per cent can be read from the graph. In the example

The volume of distribution of the drug can readily be calculated from the theoretical initial concentration and the size of the dose administered. The assumption is made that absorption was complete.

used in the body in a volume. It is of interest to know whether it is freely in solution or bound to protein. There is some evidence that it is protein bound because serum containing 15 mg/100 ml of chlor

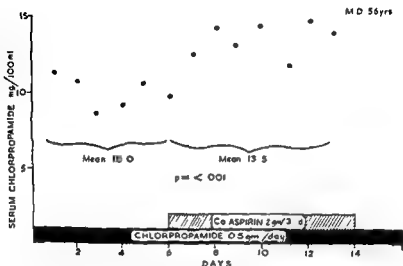


FIGURE 2 Effect of salicylate on chlorpropamide serum level

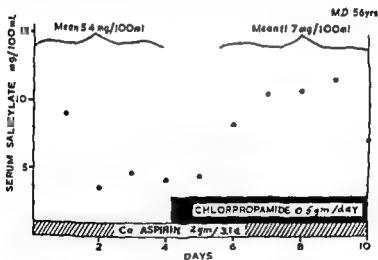


FIGURE 3 Effect of chlorpropamide on serum salicylate level

propamide had all traces of chlorpropamide removed by the precipitation of the protein with trichloroacetic acid and neither chlorpropamide nor tolbutamide has been found in the cerebrospinal fluid when the serum concentration has been as high as 14 to 24 mg/100 ml. The protein binding is evidently a loose binding because chlorpropamide can be dialyzed out of serum quite readily. The difference in the half lives of tolbutamide and



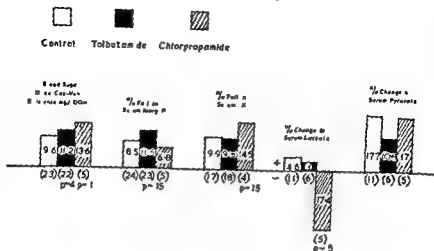


FIGURE 4 Changes during glucose tolerance tests (50 gm per os) in mild diabetes. Numbers are shown in brackets.

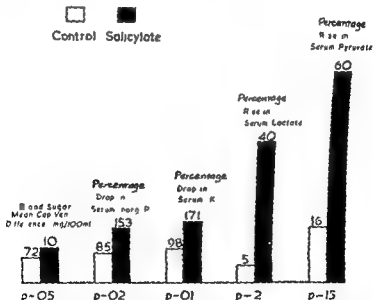


FIGURE 5 Changes during glucose tolerance tests (50 gm per os) in 11 mild diabetes.

chlorpropamide must depend largely on their handling by the kidneys, since the urine is the vehicle of their elimination. The protein binding itself would tend to limit the renal excretion of those drugs, but the mechanism of tubular transport must be the key to the understanding of this problem. Some interesting sidelights were thrown on it by studying the interaction of chlorpropamide and salicylates<sup>2</sup> on blood levels, first when given singly and

then when given together to the same individual (FIGURES 2 and 3). Such results are explained most easily by mutual interference with some process of tubular secretion in the kidney, and may have some practical bearing on therapy, since their hypoglycemic effects can be shown to be additive.

In comparing the hypoglycemic potency of tolbutamide and chlorpropamide, the superiority of chlorpropamide is apparent notwithstanding the small number of cases that we have properly studied (TABLE 2). At blood levels that were slightly lower, chlorpropamide produced a 61 per cent fall in the fasting venous blood sugar compared with a 29 per cent fall induced by tolbutamide. Indeed, so great is the hypoglycemic potency of chlorpropamide that we have twice been troubled by hypoglycemia induced in mild diabetics, a feature we have rarely experienced with tolbutamide. Unlike

TABLE 2  
HYPOGLYCEMIC EFFECT OF TOLBUTAMIDE AND CHLORPROPAMIDE  
Mild diabetics (numbers in parentheses)

	Mean percentage fall in fasting blood sugar		Mean drug level mg/100 ml
	Drug day	No treatment day	
Tolbutamide	29	(25)	12.4
Chlorpropamide	61	(5)	11.4
$p \sim 0.005$			

our experience with tolbutamide, we have never found chlorpropamide to be even probably responsible for dyspepsia. We have had three patients who had had dyspepsia, one an actual case of hematemesis that we attributed to tolbutamide. All three were changed over to chlorpropamide and made no further complaints about their digestion. The less frequent administration of chlorpropamide in itself is no doubt helpful in this respect.

Data have been obtained on patients taking tolbutamide and chlorpropamide during oral glucose tolerance tests and compared with similar data obtained when they received no treatment. In this way it was hoped that some information might be gained regarding the method of the hypoglycemic action shown. The parameters measured were the differences between the capillary and venous blood sugar levels,<sup>2</sup> the fall in the serum inorganic phosphate<sup>4</sup> and potassium,<sup>5</sup> and the change in the serum level of lactate<sup>6</sup> and pyruvate,<sup>7</sup> all estimated six times during each glucose tolerance test. The results are expressed in FIGURE 4.

If there had been an insulinlike action in the peripheral tissues characteristic changes would have been found in each of these different measurements. In fact, no significant changes were observed and thus no evidence was obtained that these sulfonyleureas acted like insulin in the muscles. The fact

\* Internal standard flame photometry.

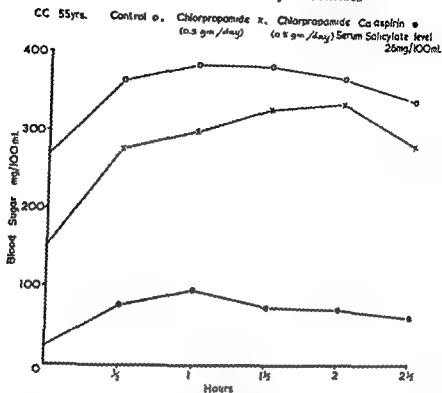


FIGURE 6 Glucose tolerance curve obtained in the same patient when on no treatment, when receiving chlorpropamide (one half gm/day), and when receiving the same dose of chlorpropamide with calcium aspirin sufficient to bring the serum salicylate level to 26 mg/100 ml

that relatively they have much more effect on the fasting than on the post-absorptive blood sugar levels suggests that they inhibit glucose output by the liver

(FIGURE 5)

significant,

TABLE 3  
BLOOD GLUCOSE LEVELS IN 4 MILD DIABETICS  
Glucose Tolerance Tests 50 gm per os

	Fasting venous blood glucose mg/100 ml				Peak venous blood glucose mg/100 ml			
	MD	MA	HC	CC	MD	MA	HC	CC
Patient Control	248	180	167	209	343	323	269	385
Sulfonylurea	62	71	120	151	189	235	209	340
Ca aspirin	121	169	143		255	347	233	
Sulfonylurea + Ca aspirin	94	45	46	22	203	297	129	96

acting like or by means of insulin. FIGURE 6 and TABLE 3 illustrate how salicylates sometimes can enhance the hypoglycemic effect of the sulfonylureas. The serum level of salicylates necessary to achieve this action is no higher than 10 mg/100 ml in some of the cases we studied.

### References

- 1 STOWERS J M, R F MAHLER & L B HENDER 1958 *Lancet* 1 278
- 2 TRINDER P 1954 *Biochem J* 57 301
- 3 HOGGETT A St G & D A NELSON 1957 *Lancet* 2 306
- 4 FISKE C H & A SHERBROW 1925 *J Biol Chem* 66 15
- 5 BARKER S B & W H SCHWEPSCON 1941 *J Biol Chem* 133 535
- 6 FREEDMAN T I & G L HUNTER 1943 *J Biol Chem* 147 415

## ONE YEAR OF CLINICAL EXPERIENCE WITH CHLORPROPAMIDE IN MORE THAN ONE HUNDRED DIABETIC PATIENTS

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During the past 13 months we have treated 130 diabetic patients with chlorpropamide the duration of treatment varying from 1 to 13 months and averaging 6.3 months

### *Method*

Our only criterion for selection of patients for a trial of chlorpropamide therapy was the presence of diabetes in a reasonably cooperative patient. The presence of common diabetic vascular complications did not preclude a trial of this drug unless they were acute nor did the occurrence of vascular complications result in a change in therapy as long as the patient remained able to take the drug. Chlorpropamide therapy was never begun while the patient was clinically acidotic however the drug was tried later in several patients who were acidotic when first seen. The majority of the patients were hospitalized for initial therapy.

Most patients were started on the following therapy with minor variations in individual cases. If the patient was on a long acting insulin this was discontinued abruptly and he was started on 0.5 gm of chlorpropamide daily usually in divided doses. During the initial period of treatment the more acute cases were given regular insulin as indicated by glycosuria. Revisions of chlorpropamide dosage were made upward or downward as necessary 5 to 7 days usually being allowed before revision upward due to the accumulation noted in the use of this drug. The dosage was gradually increased to tolerance or adequate control this being normoglycemia 75 per cent of the time and the absence of significant symptoms or side effects.

We have attempted combined therapy on but one patient. No attempt has been made to evaluate this form of treatment.

Urine analysis, liver function and hematological studies as well as blood sugar determinations were followed frequently during the initial treatment and approximately once monthly on chronic treatment.

### *Results*

Before analyzing our results it is necessary to comment on the patients who compose our series. Approximately two thirds of our patients were inmates of a state mental institution. As a group these patients constitute a poor risk for any study. There are a number of reasons for this including advanced age the average age of our entire series being 61.1 years. Most of these patients have mental deterioration that seriously limits their ability to cooperate in such matters as diet. The mental changes in many of these patients have been due to previous vascular disease of the central nervous

system, renal insufficiency, or uncontrolled diabetes itself. Many of these patients have had previous frank episodes of cerebral, myocardial, or peripheral vascular insufficiency.

Of 130 patients, 108 (83 per cent) were considered adequately controlled on chlorpropamide, 83 (64 per cent) are still under study. In diabetics previously on less than 60 to 80 units of insulin per day there was a small correlation between prior insulin dosage and likelihood of control with this drug alone (TABLE 1). There was a small but significant correlation between

TABLE 1  
(CORRELATION OF PRIOR TREATMENT WITH RESPONSE TO CHLORPROPAMIDE\*)

Prior therapy	Total cases	Response to chlorpropamide	
		Adequate control	Inadequate control
No treatment	17	17	0
Insulin			
0 to 20 units	23	21	2
21 to 40 units	41	36	7
41 to 60 units	39	16	3
61 to 80 units	9	4	5
Over 80 units	9	3	1
Unknown amount	7	4	2
Tolbutamide			
0 to 1.0 gm	9	9	0
1.5 to 2.0 gm	7	5	2
Over 2.0 gm	3	2	1
Total	146	121	25

\* Sixteen patients received both insulin and tolbutamide.

duration of diabetes and probability of control. After a duration of 10 years the patient was less likely to respond to chlorpropamide with adequate control (TABLE 2). There was a slight but positive correlation between age at the time of onset and probability of control, the older patient being the better candidate (TABLE 3). One hundred and eleven of our patients were above 40 years of age at onset of diabetes, and 86 per cent were adequately controlled. Only 2 of our patients were juvenile diabetics, and both of these cases failed to respond.

Of 4 patients previously poorly controlled on tolbutamide, 3 showed improved control on chlorpropamide. Only 4 patients poorly controlled on chlorpropamide were given a trial of tolbutamide, which also failed. All 12 patients previously controlled satisfactorily with tolbutamide were also adequately managed with chlorpropamide.

No extensive comparative studies between tolbutamide and chlorpropamide have been undertaken. However, the following three observations appear reliable. First, chlorpropamide is about 3 times more potent than

tolbutamide on a weight to-weight basis. Second hypoglycemia can be produced with chlorpropamide in most patients, this is uncommon with tolbutamide. Finally, the onset of action of chlorpropamide is approximately 50 per cent slower than with tolbutamide, however, the observed cumulative effect with chlorpropamide is more lasting than with tolbutamide. This effect has been noted for as long as 7 days following the discontinuance of chlorpropamide.

TABLE 2  
CORRELATION OF DURATION OF DIABETES WITH RESPONSE TO CHLORPROPAMIDE

Duration of diabetes (years)	Total cases	Response to chlorpropamide	
		Adequate control	Inadequate control
0 to 1	20	20	0
2 to 3	21	19	2
4 to 5	42	34	8
6 to 10	36	28	8
11 to 20	9	6	3
20	2	1	1
Total	130	108	22

TABLE 3  
CORRELATION OF AGE AT TIME OF ONSET WITH RESPONSE TO CHLORPROPAMIDE

Age at time of onset (years)	Total cases	Response to chlorpropamide	
		Adequate control	Inadequate control
0 to 20	2		2
21 to 40	17	12	5
41 to 80	111	96	15
Total	130	108	22

After initial control there appeared to be a gradual reduction in the dosage required for adequate management. It has been possible to discontinue the drug entirely in 5 patients after initial control; this could represent spontaneous or induced remission. At the time these patients were admitted to the series, treatment in addition to diet appeared to be indicated. Four of these patients had been on the following treatments: 60 units NPH insulin daily for one year; 30 units NPH insulin daily for 6 years; 15 units NPH insulin

lin for 12 years followed by tolbutamide (0.5 gm) for 6 months and tolbutamide (3 gm/day) for 8 months. The fifth patient was a newly discovered untreated diabetic. These patients will be watched with interest.

Forty six per cent of our patients on maintenance therapy require 250 mg or less of chlorpropamide daily. Seventeen per cent require 250 to 500 mg/day. 33 per cent require 0.6 to 1.0 gm/day and only 4 per cent require more than 1 gm/day.

In our series there were no untoward hematological effects. Several of our patients had mild anemias both prior to and during treatment. These conditions did not seem to be affected by the drug. Low normal white cell counts were seen with some frequency, rarely dropping below this level. There was no instance of agranulocytosis. One patient had a mild chronic lymphatic leukemia that was present prior to treatment and asymptomatic.

There were no significant renal effects. Many of the patients had albuminuria both prior to and during treatment. Most of these were thought to be manifestations of diabetic nephropathy and were apparently unchanged by therapy.

Weakness unrelated to hypoglycemia was a complaint of several patients early in the course of the treatment. This was not of such severity as to require discontinuance and subsided in a few days.

One patient developed a jaundice that was thought to be induced by chlorpropamide. This individual developed a rather severe skin reaction and eosinophilia. The drug was discontinued because of the skin reaction. Jaundice was apparent the next day. Laboratory studies suggested primarily obstructive type jaundice. Both jaundice and skin reaction cleared within a month after the drug was withdrawn. After 3 challenge doses of 0.25 gm/day there was a dermatitis and the drug was discontinued. Although it was unsubstantiated we feel this was possibly a hypersensitivity type of hepatic reaction.

Three other patients developed jaundice while on chlorpropamide therapy. In one patient the drug effect was eliminated by surgical correction of an obstructing lesion; this patient has subsequently been maintained on treatment. In another patient the drug effect was eliminated by readministration and continued treatment. The third patient developed a jaundice associated with biliary tract disease and frank acute pancreatitis. The drug was discontinued during the period of treatment for pancreatitis. The pancreatitis and jaundice cleared completely and the drug was resumed. The patient has remained clear for 2 months after chlorpropamide was resumed. Tran

#### forms of treatment

The drug was discontinued in a total of 47 patients. Seventeen of the 47 patients had the onset of a terminal illness while under study. Autopsy was performed in those cases in which the cause of death was not clinically obvious. No death was thought to have been related to the drug. The causes of death were generally those expected in elderly diabetics. Nine



were from cerebral vascular thrombosis or hemorrhage, and there were 3 episodes of fatal pulmonary emboli. Two deaths were due to myocardial infarction with failure. There was 1 death due to each of the following: staphylococcal pneumonia with lung abscess, staphylococcal pneumonia, and diabetic nephropathy with uremia. Special attention was paid to the liver at autopsy in patients who were on the drug at time of death. No histological change was noted.

The drug was discontinued in 9 of the 47 patients due to poor control. Eight of these patients are labile diabetics and are also poorly controlled on their present insulin regime in spite of our best efforts in that direction. Seven patients were lost to follow up. These essentially represented patients who manifested psychiatric recovery and were thereby discharged from the institution. As previously mentioned, it was possible to discontinue the drug in 5 patients because of apparent remission of the diabetic state. Gastrointestinal side effects, especially nausea and vomiting, necessitated discontinuance in 5 patients. Several other patients manifested lesser degrees of nausea which gradually subsided with continuation of treatment.

Chlorpropamide was discontinued in 4 of the 47 cases due to severe skin reactions that did not subside on antihistaminic treatment and continuation of the drug. These reactions subsided on withdrawal of chlorpropamide, and the role of the drug was substantiated by a recurrence with a second course of the drug.

In addition to the 130 diabetic patients, 6 nondiabetic chronic schizophrenics were given 0.5 gm. chlorpropamide daily 4 days weekly for 6 weeks in limited metabolic studies. Four of these patients suffered early morning hypoglycemia in spite of midnight feedings. This was the only significant observation. No effect on the psychiatric state was noted.

### *Summary and Conclusions*

(1) Chlorpropamide has been given to 130 diabetic patients for an average of 6.3 months, 83 per cent of these cases were adequately controlled on this medication.

(2) Side effects were those usually noted with drugs of the sulfonamide group and occurred in some 12 per cent, requiring discontinuance in 8 per cent. Nausea, vomiting, and skin reactions were the most commonly noted side effects. One patient manifested a jaundice possibly caused by hypersensitivity to the drug.

(3) Chlorpropamide is a potent hypoglycemic agent in a large percentage of nonjuvenile diabetic patients as well as nondiabetic individuals.

(4) We feel that chlorpropamide is a significant addition to our armamentarium in the treatment of diabetes mellitus.

# THE CORRELATION BETWEEN ORAL DOSAGE, BLOOD LEVELS, AND CLINICAL AND METABOLIC ACTIVITY OF CHLORPROPAMIDE IN THE TREATMENT OF DIABETIC MELLITUS\*

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Beth Israel Hospital Boston, Mass.*

Chlorpropamide was administered over a period of 3 months to 60 adult diabetic patients. These were all private patients among whom were a considerable number sufficiently reliable in their adherence to diet to be suitable for experimental trial. Treatment was combined with insulin and other hypoglycemic agents in 12 of the cases. Follow up visits were made at frequent intervals, both blood sugar and blood chlorpropamide level determinations being made at each visit. The objective of the study was to determine the therapeutic effectiveness of chlorpropamide and to ascertain the relationship between dosage, blood levels, and clinical action.

## Method

The patients had previously been treated with diet alone, with insulin, or with tolbutamide. Some had also been treated with carbutamide and DBI. As in the case of tolbutamide, the drug was used to replace all previous medication where possible. In two patients it was supplemented with insulin when necessary.

The patients were all on a nonweighed diabetic diet and returned at intervals of 3 days to 1 month. The blood sugar level was determined 1 hour after breakfast or lunch on each occasion by a picric acid method that yields results equivalent to those of the Folin Wu test. Blood chlorpropamide levels were determined by a spectrophotometric method.<sup>†</sup> The dose of chlorpropamide was deliberately varied widely in a large number of patients, usually over the range of 50 mg. to 600 mg./day, but in a few instances up to 1.5 gm./day. The total amount was administered as a single dose after the evening meal. In some instances the drug was omitted for trial periods.

Of the 60 patients, 34 had enough multiple determinations of blood chlorpropamide levels to form the basis for an analysis of the relationship between oral dosage and blood level of the drug. In all, 214 determinations were obtained for this purpose, each after at least one week or more at a given dosage level.

A composite curve of all the determinations (FIGURE 1) shows a direct proportion between dosage and blood level of chlorpropamide. The regression curve shows that for the range of dosage between 50 mg. and 1000 mg./day, each 100 mg. increase in dosage of the drug raises the blood level

\* The work reported in this paper was supported by the Chemical Research Department, Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

† Developed by the Chemical Research Department of Chas. Pfizer & Co., Inc.

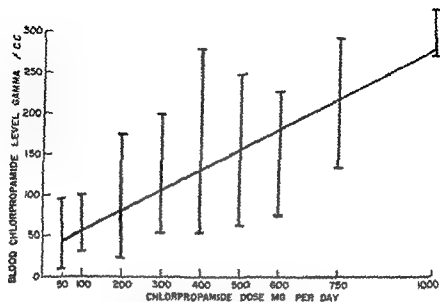


FIGURE 1 Regression curve of relationship between dose and blood levels of chlorpropamide 34 diabetic patients 214 determinations

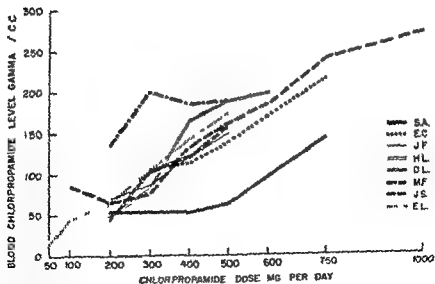


FIGURE 2 Individual patient blood chlorpropamide responses to oral therapy 8 patients at 4 dosage levels

by roughly 25 gamma/cc. In FIGURE 2, where the individual responses for

blood chlorpropamide responses

The variability of the blood chlorpropamide levels was analyzed in 10 patients with 14 different patient dosage levels. The doses studied varied

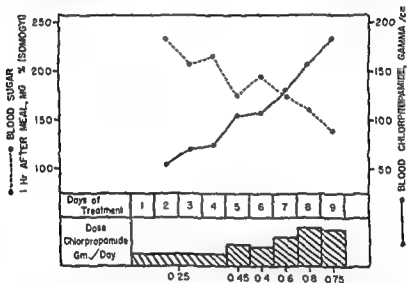


FIGURE 3 Diabetic patient L.D. acute effect of increasing dosage of chlorpropamide upon blood sugar and chlorpropamide levels over a 9 day period

from 0.2 to 1.0 gm./day, and there were 4 to 10 blood determinations at each dosage level over a period of 1 to 5 months. The variability of blood chlorpropamide levels between patients was obviously greater than those for a given patient at a given dosage level. In the latter the blood levels varied over only a limited range for long periods of time.

The wide variation in dosage of chlorpropamide administered to some 25 diabetics made possible an analysis of the relationship between the dose of drug and pharmacological and clinical effect. This was done especially for the purpose of determining the area of dose dependency of the drug. The diabetics studied were usually those of moderate severity (requiring up to 40 units of insulin daily), in whom the needed insulin could be replaced completely by chlorpropamide.

FIGURE 3 illustrates a subacute experiment. A 64 year old white female (L.D.) with diabetes of 10 years duration had previously been regulated on tolbutamide alone (1.0 gm./day). Under hospital conditions, with fixed

diabetic diet, chlorpropamide was given in increasing doses over a 9 day period. There is a striking effect on blood sugar, with rising dose and blood level of chlorpropamide. While such an acute experiment is interesting, slower increments of dosage are necessary to establish quantitative relationships between dose, blood level, and hypoglycemic effectiveness.

Accordingly, the remaining patients were studied over periods of months with slow stepwise changes in the dose of the drug. As in the case of the other sulfonylureas, the end results of replacement therapy in diabetics can be scored in terms of the blood sugar level achieved one hour after a meal as

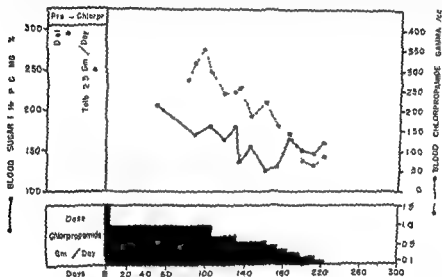


FIGURE 4 Diabetic patient J.S. a long term study, uniformly good therapeutic effect over a wide range of successively decreasing dosage

follows: fair or partial regulation, 190 to 250 mg per cent (Folin Wu), good, 150 to 190 mg per cent, and excellent, below that level.

Even in the less responsive patients with an end result of only partial regulation the maximum effective dose is still no greater than 0.6 gm day with a corresponding average blood sugar of 150 gamma per cc.

In the more responsive diabetics increase in dosage over a range of 50 mg up to 0.6 gm day may or may not improve regulation from good to excellent. When such improvement did occur it frequently could be correlated with a detectable level of dose and blood level of chlorpropamide. The reverse was true with good regulation at the higher levels, although symptoms were produced, as discussed below.

In the more responsive diabetics increase in dosage over a range of 50 mg up to 0.6 gm day may or may not improve regulation from good to excellent. When such improvement did occur it frequently could be correlated with a detectable level of dose and blood level of chlorpropamide. The reverse

could be demonstrated on the reduction of dosage level from the effective levels

In some of the most responsive patients not necessarily the mildest in

In several such cases the drug was omitted and the resultant rise in blood sugar affirmed the activity of such small doses

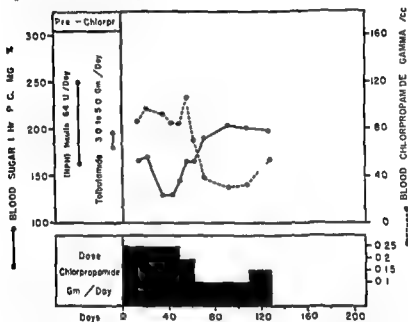


FIGURE 5 Diabetic patient J G a long term study successful replacement of 64 units of insulin and clinical titration of minimum effective dose and blood level of chlorpropamide

The following cases illustrate these principles

Case 1 Patient J B (FIGURE 4) a 33 year old white male with diabetes of 7 years duration. He had only a partial effect on 2.5 gm. of tolbutamide per day (blood sugar 250 mg per cent Folin Wu 1 hour after meals). On chlorpropamide he had a definitely better effect. The inordinately large initial dose did not produce hypoglycemia although it did produce lethargy and other symptoms. Decrease in dose and blood levels of chlorpropamide was accompanied by no decrease in effect on blood sugar. Indeed there is a suggestion of increasing effectiveness with time in the face of decreasing

in better regulation. Even better regulation was achieved on chlorpropamide (0.25 gm/day). Titration by very gradual reduction in dose showed a definite rise in blood sugar at a dosage level of 0.1 gm/day at a blood chlorpropamide level below 70 gamma/cc. The effective replacement of so large a dose of insulin by so small a dose of sulfonylurea is striking.

Case 3 Patient F B (FIGURE 6) a 79 year old female diabetic with diabetes discovered April 1958 with an initial blood sugar of 350 mg per cent. Tolbutamide (2.0 gm/day) produced an excellent therapeutic response.

#### EFFECT OF CHLORPROPAMIDE THERAPY ON BLOOD SUGAR AND CHLORPROPAMIDE LEVELS (F B)

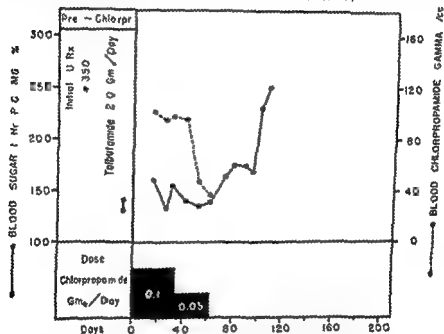


FIGURE 6 Diabetic patient F B a long term study low dosage and blood level require ment of chlorpropamide in mild diabetes proof of effectiveness by withdrawal of drug

Chlorpropamide therapy was begun in May 1958 with an initial dose of 0.1 gm/day. The response was maintained on reduction of dose to 50 mg/day and a blood chlorpropamide level of 28 gamma/cc. Omission of all therapy resulted in a gradual rise in blood sugar to 250 mg per cent over the next 40 days.

The dose dependency of chlorpropamide noted in patients studied with total replacement of insulin could also be ascertained by titration in severe diabetics in whom insulin could not be entirely omitted. In one such instance chlorpropamide (0.4 gm/day) replaced 5 units of insulin at a blood level of 159 gamma/cc while 0.6 gm/day replaced 11 units of insulin at a blood level of 210 gamma/cc.

Of the 60 patients studied several were given excessively large doses deliberately or because of the exploratory nature of the early therapeutic studies. Nine patients had definite symptoms of overdosage consisting of subjective muscular weakness a groggy or fuzzy feeling lassitude numbness and tingling in toes and nausea. All managed to perform their mental tasks although with an overlay of lassitude and sleepiness. Ataxia occurred in one exceptional individual who received 3.0 gm/day.

The daily dosage levels associated with such symptoms were 1 patient 3 gm, 2 patients 1.5 gm, 3 patients 1.0 gm, 1 patient, 0.75 gm and 1 patient 0.4 gm. There was a definite correlation with blood chlorpropamide level which was usually over 250 to 275 gamma/cc at the time of symptoms. This was underscored by the exceptional patient who had symptoms at a dosage level of 0.4 gm/day while due to unusually slow disposal the blood level was 280 gamma/cc. However other patients were asymptomatic at blood levels of 275 to 295 gamma/cc as were others on doses of 0.15 to 1.0 gm/day. This illustrates certain individual factors involved in the subjective symptoms of overdosage.

All symptoms of overdosage disappeared readily within 4 to 5 days or less of reduction in dosage even in the most highly overdosed individual (3.0 gm/day) with no residual symptoms.

Finally the average and usual dose of definitive regulation of all these cases was 0.1 to 0.4 gm/day so that the 0.75 to 1.5 gm dose productive of symptoms was 2 to 5 times the therapeutically necessary dose.

### Discussion

It is of considerable theoretical importance that there is a proportionality between oral dosage and blood levels (and presumably hypoglycemic activity) of chlorpropamide in treated diabetics; this is especially true in individual patients. The relationship is closest with least variability in blood chlorpropamide levels in individual patients on a given dose of chlorpropamide who may show remarkably constant blood levels for several weeks at a time. No accumulation of drug in the blood occurs even after months of therapy.

Since patients vary widely in the dose required for optimal regulation this leads to the distinct clinical advantage that the drug may be administered in varying doses without determination of blood levels. However individual patients do show variations from one another in their blood response. This helps to explain unexpected treatment failures and suggests the judicious elevation of the dose of chlorpropamide above the usual maximum (0.6 gm/day) in such patients.

The range of effective blood levels is quite wide from 25 to 175 gamma/cc. The effectiveness at the low dosage and blood levels in responsive patients is striking.

While clinical experience suggests that the other sulfonylureas (carbutamide and tolbutamide) probably also have the same dose-blood sulfonylurea response relationship this type of extensive long term study has not been performed for those drugs.



The risk of hypoglycemia is small in spite of the great clinical effectiveness of chlorpropamide. The level of overdosage is far beyond the usual therapeutic range, and its symptoms are so mild and easily recognizable as to present no clinical problems. It is of interest that one symptom, muscular weakness of the lower extremities has been produced in the experimental animal by very large intravenous doses of tolbutamide (270 mg/kg/hour), and is due to direct action on the spinal cord.<sup>2</sup>

#### Summary

Chlorpropamide has been administered to 60 diabetics over a period of 8 months. Both clinical response and blood chlorpropamide levels were measured. In general, there is a close relationship between dose and blood chlorpropamide response, but there are some individual variations among patients in such response. Patients maintained blood chlorpropamide levels within narrow limits at a given dosage level over long periods of time. There is no accumulation of the drug after long periods of therapy. The routine determination of blood chlorpropamide is unnecessary in clinical regulation of diabetics, although it may be useful in some nonresponsive patients.

The effective dosage range in the treatment of diabetics is 50 mg to 6 gm/day, and the corresponding blood levels are 25 to 175 gamma/cc. In some patients a demonstrable level of blood chlorpropamide for effectiveness may be determined. Hypoglycemia is rare, and symptoms of overdosage are mainly mild weakness. This does not usually occur below a dose of 0.75 to 1.0 gm/day, which is beyond the usual therapeutic range.

#### Acknowledgment

The statistical analysis of the data used in the preparation of FIGURE 1 was performed by Hilda Simon of the Statistical Department of Chas. Pfizer & Co., Inc.

#### References

- 1 SCHNEIDER, J. A., E. D. SALGADO, D. A. JAEGER & C. DELAHUNT. 1959. The pharmacology of chlorpropamide. *N. Y. Acad. Sci.* 74(3): 427-441.
- 2 GERSTENBERG, L., A. HASSELBLATT & G. SCHMIDT. 1957. Effects of high doses of the oral antidiabetic substance D 860 (known as Rastmone) on the neuromotor functions, especially of the spinal cord. *Arch. Exptl. Pathol. Pharmacol.* 231: 407-418.

# CLINICAL EXPERIENCES WITH CHLORPROPAMIDE, WITH SPECIAL REFERENCE TO COMPARISON WITH PREVIOUS EXPERIENCE WITH CARBUTAMIDE AND TOLBUTAMIDE

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At this writing it is about 3 years since we first used sulfonylurea preparations in Germany for the treatment of diabetics. During this period we have employed these drugs systematically and on a large scale. A short time ago I reported our accumulated experience with these new therapeutic agents. In that summary I emphasized the fact that there can no longer be any doubt that oral sulfonylurea therapy has proved to be of value in the treatment of diabetes despite the fact that the mechanism of action of these substances remains to be clarified completely. Approximately 40 per cent of all diabetics can be treated successfully by means of oral administration of these antidiabetic drugs, and the metabolism can be stabilized satisfactorily for the duration of the therapy. The number of "late failures" did not exceed 10 per cent. The incidence of undesirable side reactions was 8 per cent in the case of carbutamide (BZ 55) and less than 1 per cent in the case of tolbutamide (D 860).

Carbutamide and tolbutamide are the 2 substances that heretofore have been generally available in Germany for oral sulfonylurea therapy. Preference has been given to the latter, both in Germany and, to the best of my knowledge, in the United States also, because it does not possess sulfonamide characteristics. Therefore it is significantly better tolerated and exhibits an incidence of untoward side reactions considerably smaller than that of carbutamide.

Recently, experimental and clinical studies have been conducted in the United States on chlorpropamide, a new sulfonylurea derivative. We have been the first investigators to use this new antidiabetic drug in Germany, employing it for the treatment of diabetics in the Diabetic Pavilion of the Waldkrankenhaus. We had available information supplied by the manufacturer concerning the physical and chemical properties of chlorpropamide, its intensive hypoglycemic action in the dog and rat, and the observed lack of damage to the internal organs of these test animals.

## *Materials and Methods*

Altogether, we have completed control with chlorpropamide in 50 hospitalized diabetics. In an additional 24 cases, full metabolism adjustment has not yet been completed. While this is an admittedly rather small test group, the patients were very carefully examined and specific tests were conducted to determine the effect of therapy on carbohydrate metabolism and to detect any untoward effect which might develop. All patients received thorough examinations at intervals of several weeks, beginning with

the initiation of treatment. I therefore believe that we can offer very valid conclusions on the basis of our experiences.

TABLE 1 presents the composition of our test group according to age. As may be seen, the 50- to 60 year age group clearly predominates with patients. Eleven other patients were between 40 and 50 years of age. In Germany we classify adult diabetics (those having maturity-onset diabetes) chiefly into 2 categories: first, the sthenic type, which includes patients

TABLE 1  
AGE GROUPS OF THE DIABETICS TREATED WITH CHLORPROPAMIDE

Age (years)	Men	Women	Total
21-30	2	1	3
31-40	—	—	—
41-50	4	7	11
51-60	12	11	23
61-70	1	6	7
71-80	1	5	6
	20	30	50

TABLE 2  
DURATION OF THE DIABETES BEFORE INITIATION OF CHLORPROPAMIDE THERAPY

	Newly diagnosed	Time (years)							
		$\frac{1}{2}$	1	1½	2	3	4	5	7
Number of patients	10	3	4	3	7	6	6	6	1

appear well nourished and in whom insulin output may be considered to be normal or near normal, but who develop the disease late in life due to the effects of other endocrine glands on the metabolism of the patient, and second, the asthenic type, which includes patients who are thin, not well nourished, who suffer easily from fatigue, and whose insulin output may be considered to be deficient.

It is the sthenic type of diabetic who generally responds to therapy with oral antidiabetic agents, the asthenic type usually is not amenable to such therapy. The majority of our patients were of the sthenic type, and only a few could be considered as definitely of the asthenic type.

TABLE 2 indicates the duration of diabetes in each individual case, as shown, the minimum duration is six months, the maximum more than seven years. It would appear, however, that the duration of the disease is not as important a criterion for determining the advisability of initiating sulfonylurea

therapy This is the same impression we gained from evaluation of the other two aforementioned antidiabetic agents

What does appear to be more important is the age at which the disease first manifests itself We already knew from our experiences with carbutamide and tolbutamide that, in general, the prospects for success were better the later diabetes manifested itself after the fortieth year TABLE 3 shows this relationship in terms of our patients treated successfully with chlorpropamide three of them were cases where the disease developed before the

TABLE 3  
AGE AT ONSET OF DIABETES IN PATIENTS SUCCESSFULLY TREATED WITH CHLORPROPAMIDE

	Age (years)																			
	26	36	39	42	43	44	45	46	47	48	49	50	51	52	54	56	57	58	59	60
Men	1	—	—	1	1	1	3	1	1	1	—	2	1	1	—	1	2	1	1	—
Women	—	1	1	—	1	—	—	3	—	—	3	1	—	—	1	—	1	—	1	3

TABLE 4  
PREVIOUS TREATMENT OF THE DIABETICS WHO RESPONDED SUCCESSFULLY TO CHLORPROPAMIDE

No treatment	9
Diet alone	7
Diet and insulin	8
Diet and carbutamide	5
Diet and tolbutamide	19
Diet, tolbutamide and insulin	1
Diet and guanabeta	1

patient's fortieth year This is particularly worthy of attention The rest of the patients are distributed regularly with respect to the first appearance of the disease between the forty-second year and the sixty-seventh year

TABLE 4 shows that some of our patients had not been treated before initiation of chlorpropamide therapy In these cases the diabetes has been diagnosed very recently In others treatment consisted of dietary measures either alone or with insulin A third group had received either carbutamide or tolbutamide prior to chlorpropamide therapy This last group has the opportunity for

### Results

On the basis of our results, chlorpropamide appears to be a better antidiabetic agent than tolbutamide, namely the other

asthenic type in whom diabetes is a result of a pronounced lack of insulin, chlorpropamide seems to be just as unsuitable as the other two sulfonylurea preparations. Of course there are exceptions to this general rule, indeed, the treatment of all diabetics must be so carefully based on each individual case that one can say there is hardly any other disease that requires such strict individual attention. Aside from this, we had the impression in a few other cases that the use of chlorpropamide was possible in cases where carbutamide and tolbutamide had previously failed to bring about metabolic compensation at the same dosage levels. Two cases illustrating this point are shown in

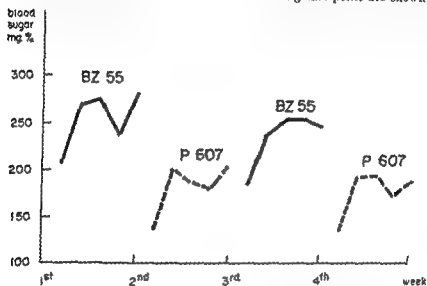


FIGURE 1. Blood sugar levels in relation to treatment with chlorpropamide and carbutamide. Patient F W H, male 53 years old. Dosage was 1 tablet twice daily (1000 mg daily).

FIGURES 1 and 2. In FIGURE 1, one carbutamide tablet twice daily, for example, was insufficient to maintain the metabolism in balance, the daily blood sugar profile showed values as high as 280 mg per cent. In contrast, 1 chlorpropamide tablet twice daily maintained blood sugar values at considerably lower levels, never exceeding 200 mg per cent. FIGURE 2 shows a similar situation in regard to tolbutamide as compared with chlorpropamide therapy.

I should also like to call your attention to 2 other points. First, when equivalent dosage of the antidiabetic agents is administered, the hypoglycemic action of chlorpropamide is definitely more potent than that of tolbutamide. Second, the fasting blood sugar, especially, is lower when chlorpropamide is administered. We observed these effects quite frequently in our test patients, and thereby we were able to come to important conclusions regarding dosage for chlorpropamide.

At the beginning of our tests we generally used an initial dosage of 500 mg of chlorpropamide three times daily. This dosage was never exceeded, and

we continued to administer it until a definite improvement in metabolic compensation was evident, with urine sugar and blood sugar values approaching normal. As our experience accumulated, we learned that for a number of patients an initial dose of 500 mg twice daily would achieve the desired result and, in a few cases, we were able to initiate therapy with a single daily dose of 500 mg of chlorpropamide. This was possible, however, in only a small proportion of the total cases. Furthermore, the initial dosage, as well as subsequent maintenance dosage, required adjustment to individual need,

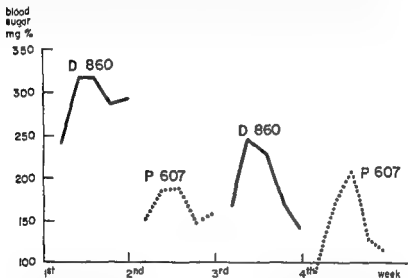


FIGURE 2 Blood sugar levels in relation to treatment with chlorpropamide and tolbutamide Patient M W, female, 57 years old Dosage was 1 tablet twice daily (1000 mg daily)

to the individual response of each patient, to the tempo at which the compensation of the metabolism progressed, and to other such factors. Thus, as with all antidiabetic medication, over all indications can be given for dosage, but no hard and fast precise directions can apply to all individuals. In a number of our patients we observed that satisfactory metabolic compensation could be obtained only if the initial dosage was 500 mg three times a day administered for several days.

With occurrence of satisfactory metabolic compensation the initial dosage was gradually reduced after 4 to 6 days, being replaced by a maintenance dosage of 125 to 1000 mg/day, as shown in TABLE 5. Few patients required a daily maintenance dosage as high as 1000 mg, and these were mostly cases whose sugar metabolism had not been maintained in balance by the same dosage of carbutamide or tolbutamide. In some of these cases examination a few weeks later revealed that metabolic compensation had improved to such an extent that the daily maintenance dosage could be reduced to 500 mg

or in some cases even to 250 mg. According to my recollection this is an observation we scarcely ever encountered during our earlier investigation of tolbutamide. With some reservations one can perhaps conclude from this observation that under chlorpropamide medication the poorly functioning pancreas improves somewhat and again begins to produce insulin in more adequate amounts.

From the facts I have already presented it is clear that it is still somewhat premature to make any definitive statement concerning the maximal initial and maintenance dosages of chlorpropamide. As I have stressed this must be a matter of individual adjustment. Nevertheless we do have the impression that the maintenance dosage is frequently considerably lower than with

TABLE 5  
MAINTENANCE DOSE OF CHLORPROPAMIDE

	Chlorpropamide (mg)					
	1000	500	250	250 every second day	125	125 every second day
Number of patients	9	14	13	1	6	1

carbutamide and tolbutamide so that in many cases the sugar metabolism can be balanced satisfactorily as long as chlorpropamide maintenance dosages on the order of 125, 250 or 500 mg are continued. Only conjectures can be offered for explanation of this behavior. Chlorpropamide is absorbed very quickly from the gastrointestinal tract. On the other hand the substance is eliminated from the body very slowly. Because of these factors it is possible to preserve a blood level of chlorpropamide high enough to accomplish a satisfactory hypoglycemic action with very small daily dosage. It is also possible that the explanation of the more potent hypoglycemic effect of chlorpropamide lies in the same factors. All of these questions require further investigation. As I have already indicated we were impressed throughout our study by the fact that the fasting blood sugar frequently was quite low. For this reason we have preferred to administer the drug early in the day and according to patient need giving either a daily dosage of one half or one full tablet immediately after breakfast as a single dose or where daily dosage is required of 1 tablet twice daily that is, after breakfast and after lunch.

Patients previously treated with insulin can be successfully placed on chlorpropamide therapy just as is the case with carbutamide and tolbutamide. With such patients we were able to replace daily insulin dosage as high as 48 units with chlorpropamide. One of these cases is shown in FIGURE 3. Heretofore the patient had taken 37 units of insulin daily. Five hundred mg of chlorpropamide daily did not produce a complete normalization of the blood sugar values which we consider the necessary criterion for the lasting

success of oral diabetic treatment. With 2 daily doses of 500 mg each the blood sugar was completely normalized so that the use of insulin could be discontinued. It is worthy of mention that in a number of newly diagnosed diabetics who suffered from acidosis before initiation of treatment we did not use insulin at all but tested the effectiveness of chlorpropamide by administering it from the beginning as the only antidiabetic therapy given. The acidosis disappeared and the metabolism was normalized. This was a most interesting observation but it was merely in the nature of a clinical

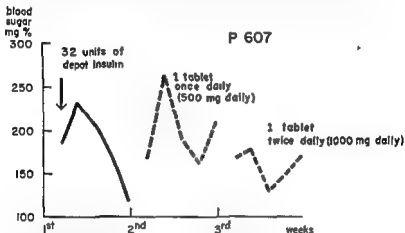


FIGURE 3 Blood sugar levels in relation to treatment with chlorpropamide and insulin. Patient W.S. male 52 years old. Dosage was as shown.

experiment and I do not recommend it as a regular procedure to be followed

returned for examination and it was found that in both cases the metabolism was decompensated. Even when the dosage was increased to 500 mg three times daily there was no improvement so that it was necessary to reinstitute insulin therapy.

#### *Side Effects*

We devoted particular attention to the question of whether chlorpropamide had any deleterious side reactions such as may be seen to some degree with carbutamide. The following may be reported up to a dosage of 500 mg

following an attack of hepatitis. Serum bilirubin amounted to 15 mg per



cent and tests of liver function gave abnormal results as expected. Heretofore the patient had been taking 40 units of insulin daily, which we replaced

chlorpropamide dose could be reduced to 250 mg. After an additional month and one half of therapy examination of the liver showed completely normal results: the liver was no longer enlarged and the function tests gave normal results. The patient felt healthy and had gained weight. This case shows not only that hepatic damage need not be feared with chlorpropamide but also that existing hepatic damage can heal during chlorpropamide treatment.

The appearance of a pruritic measleslike exanthema was noted in three patients some four to six weeks after initiation of medication with chlorpropamide therapy. This side reaction disappeared a few days after the drug was discontinued. Examination of metabolism indicated that further administration of chlorpropamide was no longer necessary, since metabolic balance could now be maintained by dietary means alone. A so called thrust effect of the sulfonylurea substances occurred, we had seen this previously when using carbutamide and tolbutamide. Furthermore, in these cases there did not appear to be a drug allergy to chlorpropamide, since the intracutaneous tests and the Prausnitz-Kustner test were negative.

Using an initial dosage of 500 mg. three times daily we observed a number of cases of gastrointestinal disturbances principally epigastric distress, feelings of pressure in the stomach and nausea. For the most part however these disappeared when the dosage was decreased so that maintenance dosages of 250 to 500 mg./day were only rarely accompanied by epigastric manifestations. Interesting in this connection is the reaction of a few patients who had also complained of epigastric distress when carbutamide and tolbutamide were used. When these patients received the smaller, but fully effective, dosage of chlorpropamide, they no longer complained of epigastric distress. Apparently they tolerated a therapeutic amount of chlorpropamide better than they did either of the other 2 sulfonylurea preparations.

### Conclusion

Again I stress most emphatically the fact that our clinical investigation of chlorpropamide is preliminary in nature and involves tentative tests conducted with a relatively small group of test patients, hence it is not feasible to come to final and definitive conclusions from this work. Further research should be conducted to establish validity in regard to indications, tolerance, side reactions and toxicity. In future investigations it will be important to explore further possible side effects that may involve the hepatic or hematopoietic systems. As I have indicated, we have never seen any sign of such effects but other investigators have reported occasional occurrences. Therefore further evaluation of this new oral antidiabetic agent is necessary.

# CLINICAL EXPERIENCES WITH CHLORPROPAMIDE GIVEN TO HOSPITALIZED PATIENTS

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## Introduction

Oral therapy for diabetes is no longer a novelty. As months have grown into years of experience with oral sulfonylurea compounds, the apprehension that harm might ensue from their use over long periods is subsiding. As a result, search for the most effective compound with the least toxicity has been intensified. This paper deals with a clinical testing of chlorpropamide in 15 hospitalized diabetic patients with a follow up of 6 months. The duration of the diabetes ranged from 1 week to 16 years (TABLE 1). Seven patients received a daily average dose of 30 U of insulin prior to the test period. One was taking 55 U daily. Two patients were receiving tolbutamide with unsatisfactory results. In 6 patients the diabetes was not under good control; none of these was receiving insulin or tolbutamide.

Ideally, the patients for chlorpropamide therapy would be selected from approximately 10 per cent of the diabetic population. This is based on the relatively sound assumptions that it is possible to control diabetes, barring acute complications, in 80 per cent by diet alone; that 5 per cent are children; and that the remaining 5 per cent are adults with labile diabetes. In these two latter groups long term treatment with chlorpropamide is ineffective.

## Method

A few preliminary days of observation preceded the test period that consisted of a minimum of 3 consecutive 5 day periods. During the first 5 days the amount of insulin given was reduced or eliminated and, in the case of patients receiving tolbutamide, this drug was withdrawn. In each instance the object was to permit hyperglycemia and glycosuria to develop in order to discern the effect of chlorpropamide more clearly. During the second 5 day period chlorpropamide was administered and throughout the third period this drug was omitted. Throughout the 15 days daily blood sugar determinations were made by the Somogyi-Nelson method on samples taken in the fasting state and at 3 M with daily quantitative determinations of the glycosuria. Tests for acetoneuria were performed on successive voidings when the insulin therapy was reduced. Other laboratory studies were made during the first and toward the end of the second week. The diet remained constant throughout and patients were guarded against undernutrition.

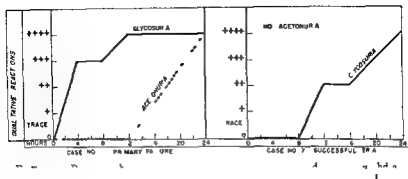
\* Research Fellow in Metabolism

TABLE I  
DATA ON CHLORPROPAMIDE THERAPY AND RESULTS IN 10 CASES OF FAVORABLE RESPONSES

Case \	Patient age & sex	Duration of diabetes	Prior treatment	Percentage fall			Chlorpropamide daily (gm)	Other
				Blood sugar		24 Hour quantity (gms)		
				Fasting	3 P M			
1	MC 66 W F	1 month	Insulin 10 U	36.3	41.5	—	0.5	
2	JR 42 W M	7 years	Diet	45.5	33.7	69.7	0.5	Tolbutamide failure
3	AW 79 W M	1 week	Insulin 8 U	47.1	31.2	37.5	0.5	
4	CS 60 W F	2 weeks	Insulin 15 U	74.1	58.2	92.3	0.25	Chlorpropamide 0.5 gm daily
5	CR 73 W F	6 months	Tolbutamide 1.5 gm	52.6	14.8	71.4	1.0	Tolbutamide failure
6	GR 76 W M	6 months	Tolbutamide 1.5 gm	57.1	40.1	85.2	1.0	Tolbutamide fair control
7	BS 57 W F	16 years	Insulin 40 U	60.5	51.9	91.2	1.0	
8	LB 68 W M	16 years	Insulin 36 U	33.0	27.4	94.8	0.75	Tolbutamide failure
9	EJ 83 W F	6 years	Insulin 44 U	44.1	24.4	—	0.75	
10	JC 51 W F	2 weeks	Insulin 55 U	4.2	11.6	46.2	1.0	
Range		1 week to 16 years	Insulin 8 to 55 U	4.2 to 74.1	11.6 to 58.2	37.5 to 94.8	0.25 to 1.0	
Average		4.6 years	Insulin 30 U	47.5	35.8	82.0	0.73	

### Results

none of these was adequate control of the diabetes as necessary. One patient who  
 if tolbutamide daily had fasting  
 cent with an average of 296 mg  
 prompt reduction of the fasting  
 blood sugar to 82 mg per cent. The comparative effects of tolbutamide and  
 chlorpropamide as observed during the period of hospitalization indicate  
 (TABLE 2) that chlorpropamide in this instance is a more potent blood sugar  
 reducing agent than tolbutamide. This impression was supported by a



In 2 cases in which tolbutamide had been a recent secondary failure excellent control of the diabetes was achieved with chlorpropamide. The drug had a blood sugar lowering effect in 10 of the 15 patients.

Following discharge from the hospital of these 10 patients the diabetes in each of them has been controlled by 0.25 to 1.0 gm. of chlorpropamide daily. The average amount given in 1 dose daily was 0.75 gm. Experience with this drug and hypoglycemic reactions caused by it have prompted us to begin therapy with a daily dose of 0.5 gm. and modify it as needed. Addi-

TABLE 2  
EFFECTS OF IDENTICAL DOSES OF CHLORPROPAMIDE AND TOLBUTAMIDE ON THE BLOOD SUGAR VALUES AND ON THE DEGREE OF GLYCOSURIA IN ONE PATIENT

	Average			6 Month follow up average & range blood sugars mg. per cent
	FBS mg. per cent	3 P.M. Blood sugar—mg. per cent	Urine sugar gm./24 hour	
Pretest control	228	284	9.8	
Chlorpropamide 1.0 gm.	147	168	2.1	194 (147-210)
Tolbutamide 1.0 gm.	184	262	11.3	296 (271-342)

tions of more than 0.25 gm to the daily dose in 1 week increased the risk of hypoglycemic reactions.

A typical favorable response is illustrated in

FIGURE 2 Both fasting and postprandial blood sugar levels were corrected. Furthermore follow-up of the blood sugar to abnormally high levels ensued.

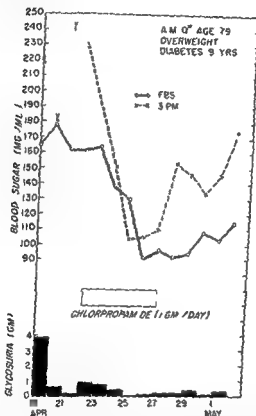


FIGURE 2 The hyperglycemia and glycosuria observed in the preliminary control period were corrected by 1.0 gm. of chlorpropamide daily. The favorable effect gradually subsided after the chlorpropamide was omitted.

### Primary Failures

Five primary failures with chlorpropamide therapy were noted. In 2 cases acetonuria appeared in increasing amounts in the first 24 hours after the withdrawal of insulin, as illustrated in Figure 1. In another case an acute cholecystitis with cholelithiasis was a complicating factor, in 1 case metastatic carcinoma was present, there was 1 patient who had gangrene of an extremity.

Data in the case of a failure to respond favorably to chlorpropamide are

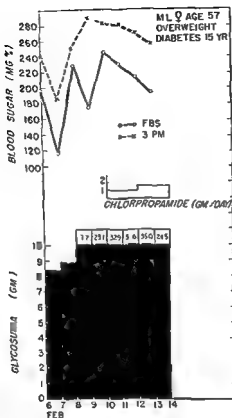


FIGURE 3 The hyperglycemia and glycosuria were not significantly improved in this case during five days of chlorpropamide therapy

shown in FIGURE 3 : Neither the hyperglycemia nor the degree of glycosuria was favorably influenced by the drug

### Side Reactions

Hypoglycemic reactions, mild in nature were accompanied in two cases

0.25 gm prevented hypoglycemic reactions

A transient pruritic, maculopapular cutaneous eruption occurred on 1 fore arm of 1 patient who had received 1 gm daily of the drug for 1 month. The rash receded under influence of antihistamine therapy and there has been no recurrence without interruption of chlorpropamide therapy

*Special Tests*

An abnormal alkaline phosphatase value was noted in one instance, but this was doubtless due to right sided heart failure with auricular fibrillation. This patient died 1 month after these studies were completed. The liver exhibited a marked central vein congestion considered characteristic of the cardiac disease, with no other detectable abnormalities. In none of the remaining 14 patients was there any detectable disturbance in the hematopoietic, renal or hepatic functions.

*Acute Effect of a Single Dose of Chlorpropamide*

The speed of action, degree of effect and the duration of the blood sugar lowering effect of a single dose of chlorpropamide were observed when a liquid dietary formula comprised of protein, 113 gm, carbohydrate, 268 gm, and fat, 63 gm (2090 calories) was divided into 12 equal amounts, 1 of which was

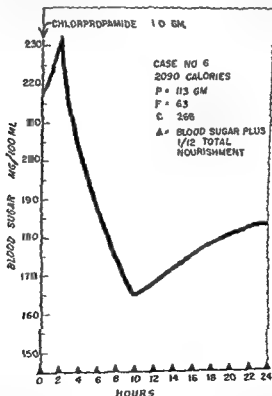


FIGURE 4 The effect of a single dose (1 gm) of chlorpropamide is illustrated. Identical liquid nourishments were given at 2 hour intervals for 24 hours. The blood sugar lowering effect became apparent between 2 and 4 hours after the first nourishment was given. This effect was most marked between 8 and 12 hours, but was still apparent after 24 hours had elapsed.

10 hours after which a slow and very gradual increase began. A marked depressing effect on the level of the blood sugar was still manifest 24 hours after the drug had been administered.

The effect of 0.5 gm given twice daily is compared in TABLE 3 with that of 1 gm given in 1 dose. The single dose produced the more consistent

TABLE 3  
DEGREE AND DURATION OF EFFECT OF 0.5 GM OF CHLORPROPAMIDE ON THE FASTING AND POSTPRANDIAL BLOOD SUGAR LEVELS AS COMPARED WITH THOSE FOLLOWING THE ADMINISTRATION OF 1.0 GM GIVEN ONCE DAILY

Chlorpropamide	Average		Urine sugar gm/24 hour
	FBS mg per cent	3 PM Blood sugar mg per cent	
0.5 gm b.i.d.	108	242	2.8
1.0 gm single dose	147	168	2.1

blood sugar lowering effect. On this basis, because of similar observations, we give a single dose of chlorpropamide before breakfast daily.

It was observed that with the daily administration of chlorpropamide its apparent effect increased for several days after which a plateau of effect ensued. Also after withdrawal of the drug the blood sugar lowering effect abated gradually over a 3- to 5-day period.

No instance of decreasing effect of chlorpropamide during therapy up to and in excess of six months has been observed. It is possible and indeed probable that the lack of secondary failures is due to the brevity of the period of therapy.

### Summary

This study of the properties of chlorpropamide involving 15 closely observed hospitalized patients indicates that this drug is a potent blood sugar reducing agent when given in doses as small as 0.25 to 0.5 gm/day. The blood sugar

the diabetes in 10 of the 15 patients having adult acquired diabetes; in no



apy, and the detection of acetonuria in increasing amounts after a reduction of the amount of insulin given served as a reliable guide in detecting those patients in whom a satisfactory response would not be obtained whereas, in cases in which no acetonuria occurred there were effective responses to chlorpropamide therapy

Prolonged therapy under close surveillance is still necessary to prove the harmlessness of this drug. Also, the likelihood must be guarded against that such an effective agent might receive widespread adoption as a substitute for appropriate dietary measures.

# OBSERVATIONS ON THE MODE OF ACTION AND CLINICAL EFFECTS OF CHLORPROPAMIDE IN DIABETES MELLITUS

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Preliminary clinical studies of chlorpropamide have demonstrated this drug to be an effective hypoglycemic agent in man. In higher dosage ranges

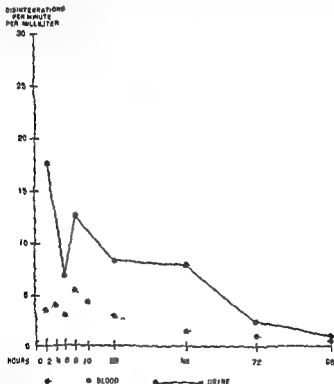


FIGURE 1 Serum and urinary levels after oral administration of 10 gm. of  $S^{35}$  chlorpropamide

it has produced marked hypoglycemia in susceptible persons. It has permitted control of clinical and laboratory abnormalities in mild to moderate

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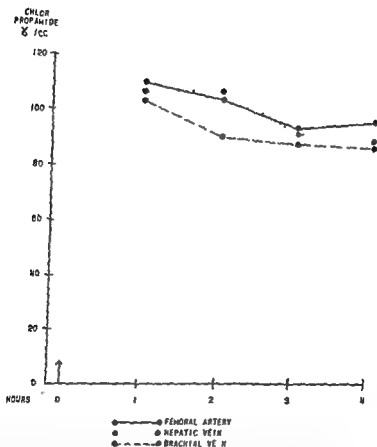


FIGURE 2 Comparison of arterial hepatic venous and peripheral venous levels of chlorpropamide after administration of 10 gm of the drug intravenously

its distribution and excretion its effects on arterial venous and hepatic vein blood sugar levels its influence on the prednisone glucose-tolerance test and epinephrine induced hyperglycemia and experience with it and other sulfonylurea derivatives on diabetes made overt by liver injury

#### Materials and Methods

Distribution and excretion were studied in 2 diabetic subjects by measuring urinary output of orally administered  $S^{35}$  chlorpropamide and in 10 non-diabetic subjects by determining drug levels in arterial venous and hepatic venous blood after oral or intravenous administration of nonradioactive

chlorpropamide The relationship of drug dosage and serum level to blood sugar changes for a 48 hour period was investigated in 6 patients with diabetes by administering 10 gm of chlorpropamide as a single dose orally Results of prolonged administration of the drug on its serum concentration were investigated by obtaining weekly determinations in 12 patients receiving 0.5 or 1.0 gm of chlorpropamide each day Comparison of arterial venous and hepatic vein drug and sugar levels was made in 10 patients by using the technique of hepatic vein catheterization<sup>3</sup> Effects of chlorpropamide on

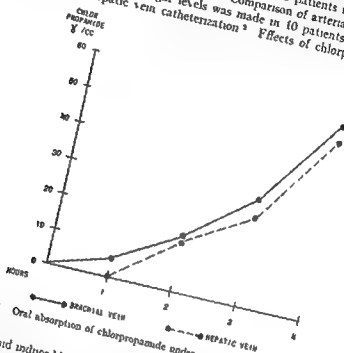


FIGURE 3  
terization

Oral absorption of chlorpropamide under condition of hepatic vein catheterization

glucocorticoid induced hyperglycemia were studied by performing a standard oral or intravenous glucose tolerance test repeating the test with prior administration of cortisone<sup>4</sup> or prednisone<sup>5</sup> and finally obtaining a glucose tolerance test after receipt of the adrenal steroid plus 10 gm of chlorpropamide The influence of chlorpropamide on epinephrine induced hyperglycemia was evaluated by studying the effects of 0.01 cc of a 1:1000 solution of intramuscular epinephrine on blood sugar before and after administration of 0.5 gm of chlorpropamide each day for 5 days Blood sugars were determined by the method of Folm and Wu Chlorpropamide was determined in the serum by an ultraviolet spectrophotometric procedure<sup>6</sup>

#### Observations

After oral ingestion of 10 gm of  $S^{35}$  chlorpropamide there was a peak rise in serum radioactivity at 4 to 6 hours followed by a gradual decrease in

serum levels and a concomitant increase in urinary radioactivity in the ensuing 48 hour period. Radioactivity was present in the serum and urine 96 hours later (FIGURE 1). A comparison of drug levels in arterial, venous and hepatic venous blood after parenteral administration of chlorpropamide showed a small but definite difference due to its uptake and metabolism by the liver and other tissues and excretion by the kidney (FIGURE 2). After oral administration of the drug in healthy subjects chlorpropamide could be

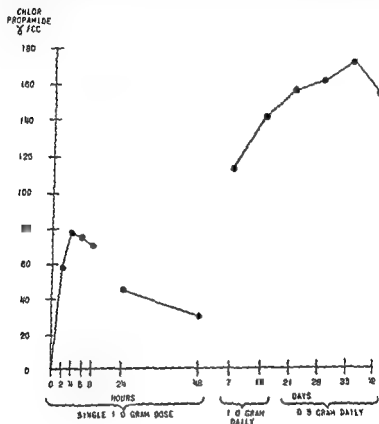


FIGURE 4: Venous levels of chlorpropamide after oral administration of the drug

detected in the pe  
presumably becau  
hepatic cirrhosis

the presence of intrahepatic and extrahepatic shunts<sup>2</sup>

Peripheral drug levels varied considerably in different subjects after a single or repeated daily doses of chlorpropamide. A level of 40 gamma or more was usually maintained for 24 hours and was often still present at 48 hours after ingestion of 1.0 gm. of the drug (FIGURE 4). Weekly drug levels in 12 ambulatory patients showed a cumulative effect of the drug with

daily administration of 1.0 gm and a similar pattern in some subjects receiving 0.5 gm (FIGURE 4)

**Blood sugar effects** Simultaneous blood sugar and drug levels after oral administration of chlorpropamide often showed an inverse relationship however the sugar response varied markedly and did not appear specifically to depend upon the height of the drug level. Although patients receiving 1.0 gm of the drug daily often had a progressive increase in serum chlorpropamide and a decrease in blood sugar there was usually a leveling off of both

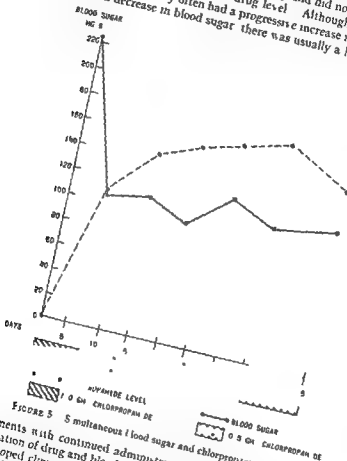


FIGURE 3 Simultaneous blood sugar and chlorpropamide levels

measurements with continued administration of 0.5 gm daily (FIGURE 5) No correlation of drug and blood sugar levels was demonstrable in 3 subjects who developed clinical evidence of hypoglycemia. Much higher chlorpropamide levels were noted in subjects who responded to this drug but did not develop hypoglycemia (FIGURE 6)

An earlier and greater hypoglycemic effect was noted when chlorpropamide was administered intravenously. The hepatic venous glucose level usually showed an earlier decrease than the arterial glucose indicating a primary

hepatic action as previously demonstrated with insulin,<sup>6</sup> phenethylbiguanide,<sup>7</sup> and other sulfonylurea derivatives.<sup>8</sup> A rebound increase in hepatic venous sugar was noted and attributed to release of epinephrine.<sup>6</sup> While a significant hepatic venous arterial glucose difference was noted, little change occurred in the peripheral arterial venous glucose difference in 6 subjects without liver disease (FIGURE 7). In contrast, 2 of 5 subjects with cirrhosis and evidence of vascular shunts exhibited a definite peripheral effect. Simultaneous chlorpropamide and blood sugar studies in 1 of these subjects showed

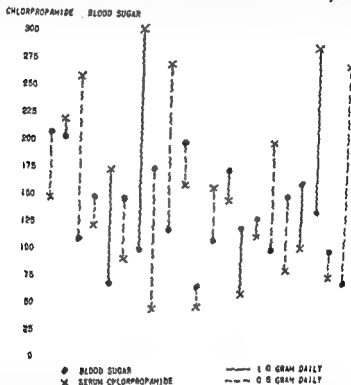


FIGURE 6 Chlorpropamide and blood sugar levels in selected diabetic patients on maintenance therapy

diminished drug uptake by the liver, little alteration in hepatic venous glucose, and increased peripheral arteriovenous glucose difference after intravenous injection of 10 gm of the drug (FIGURE 8). This phenomenon, which has numerous implications,<sup>9,10</sup> requires further study. Its selective nature is probably related to the location and number of shunts and the

hyperglycemia and glycosuria in patients with latent diabetes<sup>2</sup> or liver disease.<sup>6</sup> Studies were conducted on the influence of chlorpropamide on carbo

of an adrenal steroid was followed by an abnormal glucose tolerance test reaction) were chosen for study. In each instance chlorpropamide blocked anticipated abnormalities in carbohydrate tolerance and the post therapy curve was frequently lower than tolerance studies without adrenal steroids.

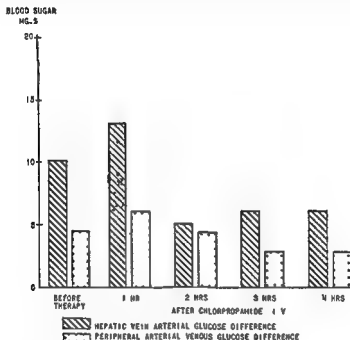


FIGURE 7 Composite of hepatic vein arterial and peripheral arterial venous glucose difference in 6 patients without liver disease after administration of 10 gm of chlorpropamide intravenously.

Similar results were obtained with oral and parenteral tests (FIGURES 9 and 10).

endogenously secreted steroids is not the basic mechanism of action of chlorpropamide; it is evident that in adequate dosage this drug prevents blood sugar abnormalities resulting from short term receipt of glucocorticoids. We have also found it effective in control of carbohydrate abnormalities in a patient with latent diabetes who required prolonged adrenal steroids for a skin disorder.



*Effects on epinephrine hyperglycemia* The capacity of sulfonylurea derivatives to increase liver glycogen has suggested their usefulness in treating patients with deficient hepatic glycogen resulting from diabetes or liver disease<sup>15</sup> Unlike healthy subjects in whom there is an increase in blood sugar of 40 mg per cent or more after administration of epinephrine or glucagon, those with liver injury and poor glycogen stores frequently show

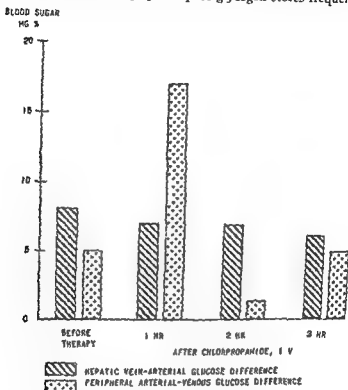


FIGURE 8 Hepatic vein arterial and peripheral arterial venous glucose difference in a patient with cirrhosis and vascular shunts

a diminution in anticipated response<sup>16</sup> In most instances there is concomitant increase in hyperglycemic response with an improvement in biochemical and histological abnormalities of the liver This improvement may be accelerated by glucocorticoids in nondiabetic patients and by insulin in diabetic patients Sulfonylurea derivatives produce a similar effect in diabetes (FIGURE 10) Evaluation of the effect of chlorpropamide in 4 subjects with chronic active liver disease, normal fasting blood sugars, and decreased glycogen stores revealed a significant increase in response to epinephrine not associated with improvement in other biochemical tests in 2 patients

*Response of liver disease and diabetes* Diabetes and hepatic dysfunction frequently coexist, and assembled evidence indicates that either of these con

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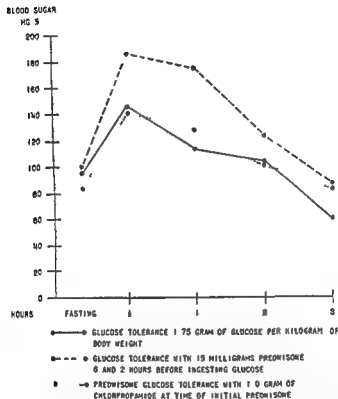


FIGURE 9 Effect of chlorpropamide on the oral adrenal steroid glucose tolerance test

of diabetics in this category have abnormal liver function tests, abnormal biopsies, or both in our experience. Only by long term serial studies is it possible to evaluate hepatic effects of this drug. To date, we have not encountered any clinical biochemical or histological changes resulting from chlorpropamide therapy.

Many have suggested that subclinical metabolic changes involving the liver are responsible for adult onset diabetes. The association of liver injury and diabetes in hemochromatosis, pancreatitis, and other metabolic and infectious processes is well documented. The diabetic may develop

intercurrent viral hepatitis biliary obstruction or hepatic changes due to heart failure however nutritional deficiency is responsible for most instances of liver disease<sup>17</sup> in this condition. The diabetic patient is similar to the insulin maintained depancreatized dog in that lipotropic deficiency produces a fatty liver and may eventually lead to cirrhosis<sup>18</sup>. In our experience diet and insulin or sulfonylurea derivatives have been effective in control of both

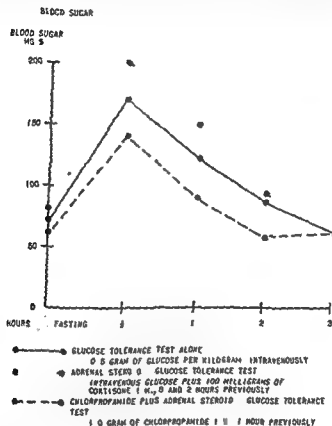


FIGURE 10 Effect of chlorpropamide on the intravenous adrenal steroid glucose tolerance test

the carbohydrate and hepatic abnormalities in most patients with liver dysfunction secondary to poor nutrition

Occasionally liver disease may be responsible for a diabetic syndrome that will disappear with improvement in biochemical and histological abnormalities<sup>19</sup>. The prednisone glucose tolerance test performed after the appearance of evidence of diabetes has permitted division of such patients into a group where post therapy adrenal steroids produce a diabetic curve and one where glucocorticoids do not alter glucose tolerance. The mechanism of the diabetic syndrome in the latter group is unknown but may be due to a dis-

turbance in regulation of blood sugar caused by interference with hepatic production or the use of humoral substances responsible for glycolysis and peripheral utilization of glucose<sup>20</sup> ■

In our experience patients with this condition readily respond to carbutamide, tolbutamide and chlorpropamide. Theoretically, sulfonylurea derivatives may be the drugs of choice in such patients because of their ease

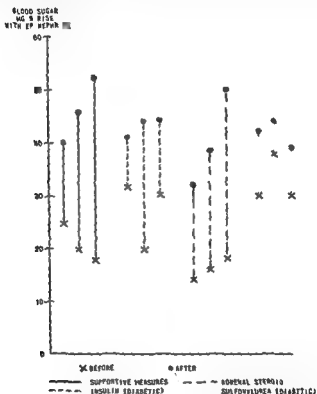


FIGURE 11 Effect of various therapeutic measures on epinephrine induced hyperglycemia in diabetes and liver disease

relationship of encountered hyperglycemia and glycosuria to liver injury. This patient was found to have hyperglycemia and glycosuria which disappeared on carbutamide and a diet of 300 gm carbohydrate, 100 gm protein and 100 gm fat. There was no history of familial diabetes or clinical and laboratory evidence of pancreatitis or endocrine disorder. An initial liver biopsy showed marked fatty metamorphosis, which disappeared after

2 months of treatment. Post therapy studies demonstrated an increase in epinephrine induced hyperglycemia over control levels. A subsequent prednisone glucose tolerance test was normal.

### Summary and Conclusions

Chlorpropamide blood levels appear to depend primarily on its absorption and urinary excretion and secondarily on tissue storage and metabolism.

This drug usually produces an earlier decrease in hepatic venous glucose than arterial glucose with little effect on peripheral arteriovenous glucose difference. Selected patients with cirrhosis and vascular shunts may exhibit a smaller than normal hepatic activity and a significant peripheral effect.

Chlorpropamide inhibits glucocorticoid induced hyperglycemia and may

respond to sulfonylurea derivatives makes it difficult to evaluate hepatotoxicity of these drugs. They have been valuable in management of both the carbohydrate disturbance and hepatic abnormalities seen in diabetic patients with secondary hepatic changes as well as diabetes made overt by liver injury.

### References

1. TORNOW A, M ZUNKE P, GREENBERG & C M LEVY. Observations on the hypoglycemic effects of N propyl N p chlorobenzenesulphonylurea in man. *Diabetes* In press.
2. LEVY C M & M GLIEDMAN. 1953. Practical and research value of hepatic vein catheterization. *New Engl J Med* 268: 696-733.
3. FAJANS S S & J W CONY. 1954. An approach to the prediction of diabetes mellitus by modification of the glucose tolerance test with cortisone. *Diabetes* 3: 296.
4. LEVY C M. 1957. Effects of prednisone on glucose tolerance in liver disease. (Abstr.) *J Clin Invest* 36: 909.
5. SCHWEIDER J A, E D SALGADO D, JAEGER & C DELAHUNT. 1959. *Pharmacology* 74: 427-442.
6. BI.
7. LI.
8. R.
9. A.
10. M.
11. W.
12. Sc.

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the action

- 13 WEST K M 1958 Relative eosinopenic and hyperglycemic potencies of glucocorticoids in man *Metabolism* 7 441
- 14 FAJANS S G L H LOUIS H S SELTZER R D JOHNSON R D GITTLER A M HENNES M L WAJCHENBERG I P ACKERMAN & J W CONN 1956 Metabolic effects of arylsulfonylurea compounds in normal men and in diabetic subjects *Metabolism* 6 820
- 15 LEEVY C M 1958 The liver in diabetes mellitus *J Med Soc N J* 55 151
- 16a KINSELL L W G D MICHAELS H A WEISS & H C BARTON JR 1948 Studies in hepatic glycogen storage. I Adrenalin induced hyperglycemia as an index of liver function *Am J Med Sci* 217 554
- 16b LEEVY C M W O'CONNELL & T J WHITE 1953 Carbohydrate disturbances in liver disease *J Med Soc N J* 50 44
- 16c VAN ITALIALE T H & W B A BENTLEY 1955 Glutagon induced hyperglycemia as an index of liver function *J Clin Invest* 34 1730
- 17 LEEVY C M C M RYAN & J A FINEBERG 1950 Diabetes mellitus and liver dysfunction etiology and therapeutic considerations *Am J Med* 8 290
- 18 LEEVY C M 1957 *Practical Diagnosis and Treatment of Liver Disease* Hoeber New York N Y
- 19 LEEVY C M J C FINEBERG T J WHITE & A M GNASSI 1952 Hyperglycemia and glycosuria in the chronic alcoholic with hepatic insufficiency *Am J Med Sci* 223 88
- 20 WALDSTEIN S S R H EYTINGER & B GIGES 1957 Abnormal glucose tolerance in experimental choline deficient fatty liver *Metabolism* 6 134
- 21 SIPERSTEIN M D & V M FAGAN 1958 Studies on the relationship between glucose oxidation and intermediary metabolism II The role of glucose oxidation in lipogenesis in diabetic rat liver *J Clin Invest* 37 1196

## CLINICAL EXPERIENCES WITH CHLORPROPAMIDE A DOUBLE-BLIND STUDY

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To determine the clinical effectiveness of chlorpropamide as a hypoglycemic agent, a double blind trial was conducted in the diabetic outpatient clinic at Pennsylvania Hospital. After completion of the trial, the patients were asked to continue in a follow up study of the drug. The response to chlorpropamide as measured by the eventual state of diabetic control during the follow up period was then compared with the results obtained during the trial and also with the control obtained with previous diabetic therapy. The results of this study indicate that chlorpropamide is a potent hypoglycemic agent that produced excellent control of diabetes in most patients.

### *Method*

The double blind study was divided into 3 periods of 2 weeks each. One week before starting, the patients stopped insulin or tolbutamide if they were taking either of these, but continued their regular diabetic diets. The first 2 week period of the trial was a control for all patients while, in the second and third 2 week periods, they received chlorpropamide, 1.0 gm daily, or a placebo. The order of receiving chlorpropamide and placebo was chosen at random. Using this sequence in the study, it was possible for a patient to have no therapy other than diet for a period of 5 weeks. In selecting patients to participate in the trial, we chose only those who, we felt, could

The patients came to the clinic in a fasting state at 8 A.M. Blood samples were drawn for a blood sugar and appropriate blood chemistries. They were then taken to a hospital dining room and were given the same 400 calorie

procedure was repeated for 6 weeks with each patient. The patients were tested their urine before meals and, at bedtime, with Tes Tape,\* recorded the results, and brought the tapes to the clinic for our count. Chlorpropamide, 1.0 gm daily, and the placebo substance were administered identically to all patients as 2 tablets before breakfast. After the trial, patients were seen at 4 week intervals in the clinic while being continued on chlorpropamide. During the follow up period, the dose of chlorpropamide was changed as indicated by the state of diabetic control. Postprandial blood sugars were not done during the follow up study.

All patients on chlorpropamide had additional routine laboratory studies

\* Eli Lilly and Company, Indianapolis, Ind

performed. These comprised a complete blood count, including hemoglobin, hematocrit, white cell count, and differential count, a routine urinalysis, direct and total serum bilirubin, alkaline phosphatase, thymol turbidity, and cephalin cholesterol flocculation. These studies were done prior to beginning the double blind trial, at the end of the trial, and routinely at 1, 2, 5 and 7 months during the follow up period, or more often if clinical indications warranted. Blood sugar determinations were made using the Somogyi-Nelson technique, while all other studies were done by the routine methods used in the blood chemistry and hematological laboratories of the Pennsylvania Hospital.

The results of the double blind study were assessed by averaging the fasting and postprandial blood sugars separately in every patient for each 2 week period. These values were then compared for the 3 periods. This permitted a comparison of both the fasting and the postprandial blood sugar values between the control and placebo periods, between the control and the chlorpropamide periods and between the placebo and the chlorpropamide periods. An adequate response was taken to be a decrease in both the fasting and postprandial blood sugars of 20 per cent or more when levels in the treatment period were compared to those obtained in placebo and in control periods.

The final state of control of the diabetes achieved during the follow up period was classified as good, fair, and poor. The criteria used were as follows: good control, fasting blood sugar under 120 mg per cent and no glycosuria, fair control, fasting blood sugar below 150 mg per cent and minimal glycosuria, poor control, all conditions worse than this. Those patients who had taken tolbutamide prior to this study also had their diabetic control while on that drug assessed in the same way so that a comparison between the level of control on tolbutamide and on chlorpropamide could be made.

### *Results of Double Blind Trial*

Thirty two patients completed the trial. The clinical characteristics of the group are given in TABLE 1. All patients were in the older age group,

TABLE 1  
CLINICAL CHARACTERISTICS OF PATIENTS IN CHLORPROPAMIDE TRIAL

Age (years)	59.8 av	45-78 range
Age onset of diabetes	52.6 av	32-75 range
Duration of diabetes	6.6 av	0-31 range
Previous therapy		
Insulin	23 patients	50-70 IU range
Diet only	6 patients	
Tolbutamide	19 patients	Just prior to trial

with an adult type of diabetes. There were 5 men and 27 women. Twenty three had taken insulin at some time and 19 were taking tolbutamide immediately prior to the trial.



The response to chlorpropamide is shown in TABLE 2. The results are divided into 2 groups: 23 patients who received the placebo in the 2-week period before chlorpropamide, and 9 who received chlorpropamide before the placebo. Thirteen, or 56 per cent of those in the first group, showed a fall in blood sugar of 20 per cent or more when the fasting and postprandial blood sugar values during chlorpropamide administration were compared

TABLE 2  
RESPONSE TO CHLORPROPAMIDE

Order of trial	No. patients	Adequate response	Inadequate response
Placebo first	23	13 (56%)	10 (44%)
Chlorpropamide first	9	8 (90%)	1 (10%)
Total	32	21 (65%)	11 (35%)

Adequate response = fall in fasting and postprandial blood sugar values of 20 per cent or greater compared to control and placebo levels.

TABLE 3  
RESPONSE TO CHLORPROPAMIDE: PERCENTAGE CHANGE OF BLOOD SUGAR

Order of trial	Placebo Control		Chlorpropamide Control		Chlorpropamide Placebo	
	FBS	PPBS	FBS	PPBS	FBS	PPBS
Placebo first						
Adequate response	+2%	+2%	-27%	-23%	-28%	-22%
Placebo first						
Inadequate response	+10%	+16%	+2%	+11%	-10%	+1%
Chlorpropamide first						
Adequate response	-22%	-19%	-33%	-31%	-11%	-10%

to the values of the placebo and the control periods. Eight of the 9 patients who received chlorpropamide first showed an average decrease in blood sugar of more than 20 per cent, but in this group the values obtained on chlorpropamide were compared only with the control values.

TABLE 3 shows the average percentage change in blood sugar values in the 2 groups. The 3 comparisons are given separately for both fasting and postprandial blood sugar levels. When placebo administration followed the control period, the blood sugar values were higher during the placebo period. However, when chlorpropamide was given before the placebo, the placebo blood sugar levels were about 20 per cent lower than the control levels. This was assumed to be due to the persistence of the hypoglycemic

effect of chlorpropamide after the drug was stopped. An adequate response in this group of patients was judged on a comparison of the control and the chlorpropamide periods only. It can also be seen that in each group the decrease in blood sugar was slightly greater in the fasting than in the post-prandial levels. However, the difference was so slight that, in a patient who has fasting hyperglycemia, determination of the fasting blood sugar alone would have been an adequate indicator of response.

### Follow-Up Study

Thirty of the 32 patients continued to take chlorpropamide after the trial was completed. Of the 2 patients not followed, 1 was hospitalized with a cerebral thrombosis 5 weeks after the trial, the other refused to return. TABLE 4 gives the final results to date for the remaining 30 patients. Twenty-five of these achieved good control, although the maximum response was

TABLE 4  
RESPONSE TO CHLORPROPAMIDE FOLLOW UP OF 30 PATIENTS

Present state of diabetic control	No. of patients	Duration until best control in weeks				
		4 or less	6	8	12	16 or more
Good FBS under 120 mg per cent No glycosuria	25	13	3	2	6	1
Fair FBS under 150 mg per cent Slight glycosuria	4	4				
Poor	1					

Of 11 patients with inadequate response during trial, 10 were available for follow up and 9 achieved good control.

TABLE 5  
RESPONSE TO CHLORPROPAMIDE COMPARISON OF CONTROL WITH TOLBUTAMIDE AND CHLORPROPAMIDE

Tolbutamide control		Chlorpropamide control		
		Good	Fair	Poor
Good	7	7	0	0
Fair	7	7	0	0
Poor	5	4	1	0
Total	19	18	1	0

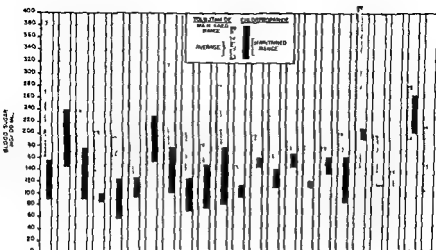


FIGURE 1

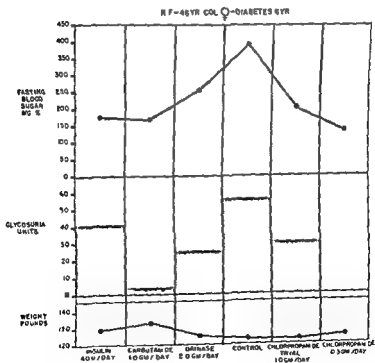


FIGURE 2

often delayed for several weeks. Probably this was due to the fact that the dose of chlorpropamide was usually reduced to 0.5 gm daily after the trial proper was completed. The 1 patient who failed to show a good response was a 45-year old Negro female with diabetes of 5 years' duration who had required 50 U of insulin daily for indifferent control before the study.

Nineteen patients were taking tolbutamide before the chlorpropamide trial started. The effectiveness of diabetic control with these 2 drugs is compared in TABLE 5. Seven patients showed good control with both drugs.

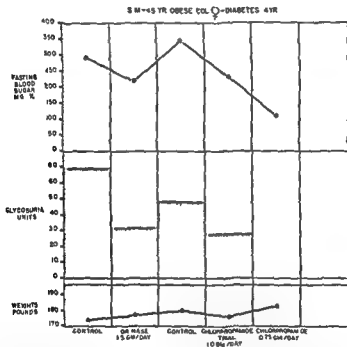


FIGURE 3

Of 12 patients not well controlled on tolbutamide, all showed improvement with chlorpropamide, and with one exception, the final state of diabetic control was very satisfactory. FIGURE 1 illustrates this by comparing the range of fasting blood sugar determinations on the two drugs. In almost every case the blood sugar values are substantially lower with chlorpropamide. There was no correlation between success or failure with tolbutamide and the dose of chlorpropamide necessary to achieve good control. FIGURE 2 presents the chronological history of a patient who was treated successively with insulin, carbutamide, tolbutamide, diet alone, and chlorpropamide. Average values for fasting blood sugar, glycosuria, and weight are given for each period of therapy. The patient showed poor control on 2.0 gm tolbut

amide daily but has been very effectively controlled with a single daily 0.5 gm dose of chlorpropamide. FIGURE 3 shows the results of oral therapy in a gluttonous woman who might be controlled by a low calorie diet and weight reduction. Neither threats nor bribes nor tolbutamide was effective in controlling her appetite or her diabetes, however on a dose of chlorpropamide that has now been reduced to 0.5 gm daily, she has an average fasting blood sugar of 100 mg per cent and no glycosuria. Her appetite is unchanged and she continues to gain weight.

### Toxicity

The side effects and possible toxic reactions noted with chlorpropamide are listed in TABLE 6. Six patients complained of symptoms suggestive of

TABLE 6  
RESPONSE TO CHLORPROPAMIDE SIDE EFFECTS AND TOXIC REACTIONS

Side effect or reaction	No of patients
Hypoglycemic symptoms	8
Fasting blood sugar below 60 mg per cent	4
Hunger	5
Gastrointestinal disturbance	0
Skin rash (transient)	2
Liver function test abnormal (transient)	3
Blood count or urine tests all normal	0

hypoglycemia and in 4 instances fasting blood sugar values below 60 mg

per cent with chlorpropamide therapy. Two patients developed a mild itching maculopapular eruption. There was no associated eosinophilia and none due to continuing

### Summary

Thirty-two patients participated in a double blind trial of chlorpropamide. All had an adult type of diabetes but every patient also had glycosuria and fasting hyperglycemia during the control period of no therapy. Twenty-one or 65 per cent of the group had a decrease in both fasting and postprandial blood sugar. Thirty patients of the 5 gm

or less daily given in a single dose. The maximum response to chlorpropamide may be delayed until 4 weeks or more after therapy is started. Comparison of diabetic control between tolbutamide and chlorpropamide in the same patients shows that good control often can be achieved with chlorpropamide when tolbutamide has been proved ineffective. Chlorpropamide was a more potent hypoglycemic agent than tolbutamide. Toxic reactions were seen in 5 patients but in every case the manifestation of toxicity subsided without stopping therapy. Hypoglycemic reactions occasionally severe may be provoked by chlorpropamide and the dosage must be individually adjusted.

#### *Acknowledgments*

We thank Brooks W. Gilmore, Joyce Laubach, and Sandra Frank for their invaluable assistance during the study.

# LONG TERM THERAPY WITH CHLORPROPAMIDE

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The influence of chlorpropamide has been evaluated in 56 diabetic patients with reference to changes in hyperglycemia, glycosuria, liver function, the hematopoietic system, and general health

## *Materials and Methods*

The subjects were selected largely from the diabetic clinic of St Boniface Hospital. As a group they tended to have little respect for dietary restrictions. Before inclusion in the study an attempt was made to categorize the diabetes as stable (adult, obese, nonketotic, and senile) or labile (juvenile, thin, ketotic, and brittle). Using this clinical assessment, we felt that 49 subjects had stable diabetes.

The initial response to chlorpropamide was assessed in hospital on 37 patients. The other 19 were studied as outpatients. The laboratory tests done prior to therapy and at intervals thereafter were a complete urinalysis, hemogram, oral glucose tolerance (Folin Wu), blood urea nitrogen, plasma prothrombin time, serum alkaline phosphatase (King Armstrong), glutamic oxaloacetic acid transaminase, phosphohexose isomerase, thymol turbidity and flocculation, cephalin cholesterol flocculation, cholesterol, and esters, and Bromsulphalein excretion. The radioactive iodine uptake by the thyroid was measured in 5 of these subjects.

Depending on the type and severity of the diabetes, chlorpropamide administration was begun in one of several ways: the drug was given and the insulin gradually reduced; insulin was discontinued 24 hours before giving chlorpropamide; insulin was discontinued for several days; patients who had not been using insulin were given the drug after a preliminary assessment on a constant diet. In all cases 1.0 gm chlorpropamide was given on the first day and 0.5 gm daily until the hypoglycemic effect could be

## *Results and Discussion*

Thirty nine of the 56 subjects have received chlorpropamide for at least 4 months, which is our criterion for long term therapy. The 7 patients adjudged to have labile diabetes all failed to show a hypoglycemic effect with chlorpropamide either alone or in combination with insulin. They were given the drug from a minimum of 4 days to a maximum of 20 days. We concluded that chlorpropamide, like the other sulfonylurea drugs, does not control the labile diabetic. However, our dose schedule for initiating therapy with chlorpropamide was not varied, and it is possible that a higher initial

TABLE I  
ANALYSIS OF PATIENTS WITH STABLE DIABETES

Age and sex	Dietary		Insulin			Chlorpropamide			Comments
	Duration (years)	Complications	Duration (years)	Insulin dose (units/day)	Control	Duration (days)	Dose (mg)	Control	
56M	New	+	0	0		7	200	0.5	G† Sudden death M. infarct
74M	2	+	Only w thacetyl cholecyst t s			10	0.5	G‡	Recurrent cholecyst t s
66M	14		0	0	F	7	0.5	‡	Obstruct ve gland cr due to Ca pancreas
70M	12	+	0	0		20	0.75	G‡	
46F	4		0	0	P	40	0.5	‡	P on 0.5 gm
67F	11	+	0	0	M	45	0.75	‡	P on 0.75 gm
56M	9	++	0	0	P	7	2.0	P	
68F	14		14	75	M	21	2.0	P	
65M	16	+	12	40	M	10-16	2.0	P	Diarrhea first course none later
66F	10	-	10	30	C	14	0.75	P	Adrenexa derm? reaction
66F	5	+	5	70	P	120	1.5	P	Hypoglycemic effect in hosp tal
43F	9	-	4	35	P	200	2.0	G	Int al success opera t on
42F	3		3	30-40	F	270	1.8	G P	Int al suc ess
72F	10	+++	3	25	P	230	1.0	G	Died post op ch ls cyste tomy
41M	11	-	1	35	G	90	0.5	G	Tr amc nalone 100 mg/day d ed leukem a
59F	19	+	0	0	F	300	0.5	C	
81F	2	+	1	15	F	180	0.25	C	
71F	7	+	1	20	F	190	0.75	P	
61F	11	++	15	30	P	180	0.5	M	Gangrenous toe
76F	2	++	14	15	G	220	0.5	G	Hyperthyroidism
66F	3		3	50	P	150	0.5	M	Cellul t s
72M	5	++	0	0	P	240	0.25	G	
63F	9	+	13	25	M	240	0.75	C	Transurethral resect on
73F	22		28	30	G	270	0.25	C	Pneumon a
71F	5	+	5	30	P	240	1.0	G	
52M	2	-	0	0	P	2.0	0.5	F	
74M	6	+	0	0	P	270	0.75	C	Flu tracheostomy therapy
49F	5	-	2	30	P	150	1.0	P	
65M	1	+	0	0	P	180	0.5	C	
68F	8	++	4	20	P	270	0.25	G	
79M	3	+	4	30	M	240	0.5	G	
50F	7	+	5	50	F	270	0.75	P	
61F	5	+	2	30	P	240	0.5	G	Myocardial infarct
49F	1	-	0	0	P	180	0.25	G	Abdom inal burn
77M	Recent	+	0	35	P	180	0.5	M	Cerebrovascular acc ident
77F	5	+	5	35	P	135	0.25	G	
70F	18	++	0	0	P	170	0.25	G	
67F	1	-	16	25	F	300	0.25	G	
55F	5	++	5	10-20	F	180	0.25	G	
74F	12	++	0	0	P	130	0.75	G	
50F	7	-	2	15	P	240	0.5	M	
63F	2	++	1	35	C	200	0.25	C	
70F	7	+	6	30	F	180	1.5	F	
67F	7	-	4	20	P	240	0.5	G	
64F	11	+	23	35	F	210	0.75	G	
73F	3	+	0	0	P	190	0.25	G	
60F		+	2	15	M	270	0.25	G	
64F	0	-	0	0	P	210	0.25	G	
65F	New		0	0		120	0.5	C	Cate n ma cul n re sected

\* Control P = poor M = fair G = good

† Chlorpropamide given in 2 courses

‡ Lost to follow up

§ At onset of diabetes



dose might have produced hypoglycemia. No side effects other than ketosis were observed. One labile patient, a 39 year-old female, died 10 days after chlorpropamide was discontinued. The cause of death was a pulmonary embolus following electroshock therapy. The consulting psychiatrist felt that her psychosis was not drug induced.

Some of the pertinent data on the 49 stable diabetics are shown in TABLE 1. The complications indicated as +++ refer to retinopathy, neuropathy, nephropathy and other occlusive vascular disease. By good control we mean

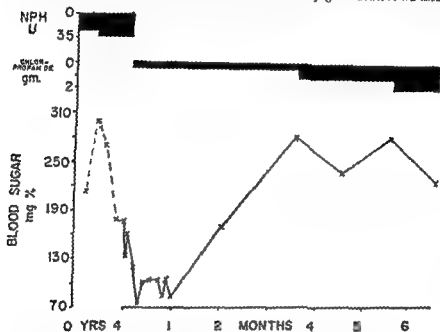


FIGURE 1 Patient H M female aged 45 diabetic for 9 years

aglycosuria and fasting normoglycemia, and by fair control we mean fasting blood sugars (Folin Wu) between 120 and 160 mg per cent. Fasting blood sugars over 160 mg per cent indicate poor control. The patients varied in age from 41 to 81 years, with a mean age of 63. Ten of these subjects did not satisfy our criterion for long term therapy. Six were lost to follow up, 3 failed to show a hypoglycemic effect on 2.0 gm chlorpropamide daily, and 1, who remained hyperglycemic on 0.75 gm daily, became anorectic and drowsy. Of those lost to follow up, 1 died suddenly of a myocardial infarct diagnosed post mortem. His initial response had been good and he may have been hypoglycemic at the time of infarction. One died of *E. coli* septicemia during his third bout of acute cholangitis. He was normoglycemic on 0.5 gm chlorpropamide between the second and third attacks. The drug was discontinued at the onset of symptoms 2 weeks prior to death. At post mortem multiple liver abscesses were found, together with common duct calculi and a contracted gall bladder. A third patient developed

1 showed good initial control on 0.5 gm and 2 showed poor initial control on 0.5 gm

All of the 39 long term subjects showed an initial hypoglycemic effect. Three were late failures. FIGURE 1 shows the decrease in fasting blood sugar level in a 45 year old obese female who was changed from insulin to

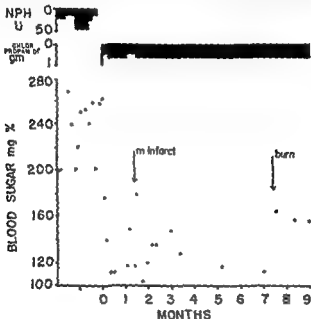


FIGURE 2 Patient D, female aged 50 diabetic for 7 years

pital but control at home was better than on previous therapy. The other 30 patients remained well controlled at home.

Two of the long term subjects have died, one of leukemia and the other of subphrenic abscess following cholecystectomy for chronic recurrent cholecystitis. Both patients showed good control on chlorpropamide. Good control was maintained on the leukemic patient during a 3 week period when he received tramcinolone (100 mg/day).

In the long term group 10 further medical events have occurred. 1 myocardial

shows the blood sugar response in a 50 year old female who had first a myocardial infarct and later a second degree scald of the abdomen. The possible role of chlorpropamide in the production of these fatal and nonfatal events is not known, but no direct correlation could be detected.

Oral glucose tolerance tests done 24 hours following therapy showed a hypoglycemic effect, but no other difference in the type of curve. FIGURE 3 shows 1 pretreatment and 3 treatment curves in a 52 year-old male. The subject was on an unrestricted diet during the 7 months between the third and fourth tests. The fourth curve shows less hypoglycemic effect.

Liver function showed no deterioration except in 3 patients who died. In those 3 a satisfactory explanation of the pathology was found (see above).

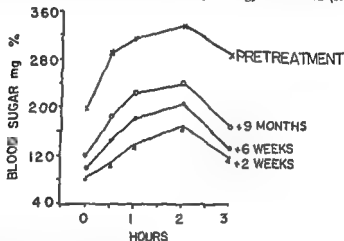


FIGURE 3 The effect of chlorpropamide on oral glucose tolerance. Patient A, a male, aged 52, diabetic for 2 years.

In 3 other patients Bromsulphalein excretion improved during drug administration. Hematological studies showed no change in hemoglobin and no evidence of agranulocytosis or thrombocytopenia. Two patients showed a transient unexplained eosinophilia. Chlorpropamide did not change the uptake of radioactive iodine by the thyroid in the 5 patients studied, including the one with hyperthyroidism.

Symptoms that may have been due to the drug were diarrhea in one patient and pruritus in another. In both cases the drug was continued, with disappearance of the complaint. One patient complained of anorexia and drowsiness with hyperglycemia but no acidosis, and the drug was discontinued.

An attempt was made to find a correlation between duration of insulin therapy and blood sugar response to chlorpropamide in the stable group. Of 18 who had never taken insulin, 10 showed a good response, 1 a fair response, and 1 a poor response. Six were lost to follow up. Of 18 on insulin less than 5 years, 14 showed a good response, 2 a fair response, and 2 were late failures. Of 13 on insulin for 5 years or more, 6 showed good control, 3 showed fair control, and 4 showed poor control. Of the 31 who

had used insulin, 5 had taken more than 40 units daily. Three of these patients showed improved control with chlorpropamide. Ten patients in the stable group had previously used tolbutamide. Of these, only 3 had improved on tolbutamide, whereas 7 improved on chlorpropamide. This

13 used 0.5 gm, 6 used 0.75 gm, 3 used 1.0 gm, and 1 used 1.5 gm daily. Final adjustments in dosage have not yet been made.

### *Summary*

We could neither control the hyperglycemia nor prevent ketosis with chlorpropamide in 7 labile diabetics. Thirty-six of 43 stable diabetics were controlled better with chlorpropamide than with previous therapy. Although the mortality and morbidity rates were high, chlorpropamide could not be implicated. No deterioration of liver, hematopoietic, or thyroid function could be determined. Diarrhea, pruritus, and anorexia each occurred in one patient. Neither the amount nor the duration of insulin therapy correlated with the hypoglycemic response to chlorpropamide. Some tolbutamide failures were controlled by chlorpropamide. The maintenance dosage of chlorpropamide averaged 0.5 gm daily.

### *Acknowledgments*

We acknowledge with pleasure the help of M. C. Blanchaer and Paul Green, staff members, St. Boniface Hospital, Winnipeg, Canada.

# CLINICAL EVALUATION OF CHLORPROPAMIDE IN DIABETES MELLITUS

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The use of the sulfonylureas for the treatment of diabetes mellitus is now well accepted. Numerous studies dealing with the indications, contraindications, and clinical results of oral treatment with carbutamide and tolbutamide have been published. Therefore, in a clinical study of a new drug chlorpropamide, 1 propyl 3-(*p* chlorobenzenesulfonyl)urea, it seems unnecessary to survey the literature, even for the sake of comparison, since the fundamental therapeutic data are so well known.

## MATERIALS

Chlorpropamide was administered to 80 diabetics (38 males, 42 females). The ages of the patients, dates of onset, duration of diabetes, nutritional

TABLE 1  
AGE AND NUTRITIONAL STATUS IN 80 PATIENTS TREATED WITH CHLORPROPAMIDE

Nutritional status	Age in years			Total	Percentage
	<20	21-40	>40		
Obese	—	4	44	48	60.0
Normal	—	5	9	14	17.5
Underweight	2	5	11	18	22.5
Total	2	14	64	80	100.0

TABLE 2  
AGE OF ONSET AND YEARS OF DIABETES IN 80 PATIENTS TREATED WITH CHLORPROPAMIDE

Age of onset	Years of diabetes					Total	Percentage
	<1	1-5	6-10	11-15	16-20		
<20	—	2	—	—	—	2	2.5
21-40	3	8	8	—	4	23	28.7
>40	6	19	12	15	3	55	68.7
Total	9	29	20	15	7	80	100.0

TABLE 3  
COMPLICATIONS OF DIABETES AND ASSOCIATED DIAGNOSES IN 80 PATIENTS TREATED WITH CHLORPROPAMIDE

Complications or associated diagnoses	No. of cases
Retinopathy	29
Nephropathy	17
Atherosclerosis	42
Blood hypertension	15
Tuberculosis	9
Other diagnoses	10
Patients with 1 or more complications or associated diagnoses	53
Patients without complications or associated diagnoses	27

TABLE 4  
THERAPY PRIOR TO CHLORPROPAMIDE IN 80 PATIENTS

Prior therapy	No. of cases	
Insulin treatment	35	61
Insulin only		
Insulin and oral treatment (nonsimultaneous)	26	
Oral treatment only		8
New cases without treatment		4
Old cases without treatment		7
Total		80

TABLE 5  
YEARS OF INSULIN AND DOSE IN 61 PATIENTS TREATED WITH CHLORPROPAMIDE

Years of insulin	Dose of insulin (un ts)			Total
	<20	21-40	>41	
<1	3	11	4	18
1-5	4	15	1	20
6-10	—	8	1	9
11-20	1	5	1	7
Not determined	3	4	—	7
Total	11	43	7	61

condition complications and associated maladies, and previous treatment are illustrated in TABLES 1, 2, 3, 4, and 5

### METHOD

Thirty four cases were first treated while hospitalized, all the other cases were outpatients. The diet, adjusted to the theoretical caloric requirements, varied between 150 gm and 250 gm of carbohydrates. The initial dosage of chlorpropamide was 2 gm (23 cases) and 1.5 gm (5 cases) daily, based on the usual dosage schedules for the other sulfonylureas. As our evaluation continued we found that we could reduce the initial dosage to 1 gm (49 cases) and 0.5 gm (3 cases) daily. In general, divided doses were employed, except during the maintenance period, when the dose was reduced to 0.25 gm daily. In the case of 2 patients, both chlorpropamide and insulin were given.

The outpatients were examined weekly at first, and then monthly. In these cases the blood sugar was determined during a fasting state and urine sugar was determined from a 24 hour pooled sample (glycemia determined by the Solomos method). Before and during the course of treatment tests were made of hepatic, hematopoietic, and renal functions (flocculation, alkaline phosphatase, prothrombin, Bromsulphalein retention, hemogram, and complete urine tests).

The first dose was administered as a single dose and the hypoglycemic action of the drug was determined in 76 cases at 3, 4, 5, and 24 hours (selection test). On the basis of these results the potency of the drug was established. The patients fasted during the first 5 hours of study. In addition to observing immediate action, we wished to determine whether the results of this test would relate to the results of previous treatment. Serum chlorpropamide also was tested during the course of the treatment.\*

### RESULTS

#### *Hypoglycemic Action of the Drug*

*Selection test.* Response as observed in the selection test was based on blood sugar determinations on samples taken 5 hours after the initial dose was administered (final glycemia). On the basis of our prior experience with other sulfonylureas,<sup>1</sup> the result was classified as good when the final blood sugar was lower than 1.60 gm per cent or a drop of more than 50 per cent from the initial figure occurred. The result was considered fair when the blood sugar was between 1.61 and 2 gm per cent or when a drop of between 25 and 50 per cent from the initial figure occurred, the result was termed poor when the final blood sugar was over 2 gm per cent or when a decrease of less than 25 per cent from the initial figure occurred.

A significant hypoglycemic effect appeared in 52 patients (68.4 per cent), the other 24 did not respond to the drug. FIGURE 1 presents the curves of the average values obtained in the patients who responded to and those who did not respond to the drug. The tests carried out with 1 and 2 gm of chlorpropamide are shown separately. The greatest decrease in blood sugar was

\* Using the technique communicated to us by Chas. Pfizer & Co. Inc., Brooklyn, N. Y.

the other sulfonylureas chlorpropamide has an immediate hypoglycemic action

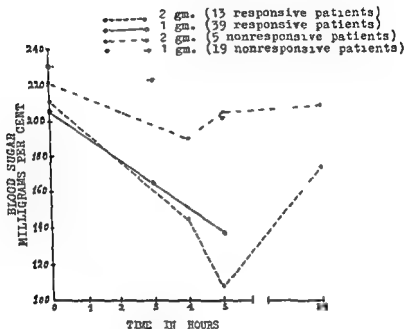


FIGURE 1 Average blood sugar response to different doses of chlorpropamide

TABLE 6 presents the results of four selection tests conducted on patients who received chlorpropamide in which serum levels of the drug were also determined at the same time

It may be seen that in the 3 cases with good hypoglycemic response there were considerable quantities of chlorpropamide in the blood during the early hours of the test. These values increased even more at 5 hours. The increase is more rapid in some cases. There seems to be a period of latency of action since the blood sugar values rapidly fall at 5 hours after 0.5 or 1 gm are administered even though in the serum at the 3 hour determinations there are greater quantities of chlorpropamide than are usually observed in patients who have responded to the drug and are receiving maintenance dosage. The case with poor response (case 4) showed serum chlorpropamide levels at 3 and 1 at 24 hours that were higher than those obtained in other patients who had a favorable response. In addition to the usual differences



TABLE 6  
CHLORPROPAMIDE SERUM LEVEL AND BLOOD SUGAR RESPONSE IN 4 PATIENTS,  
AFTER ADMINISTRATION OF A SINGLE DOSE OF 10 GM

	0 Hour blood sugar	3 Hours		5 Hours		24 Hours	
		Blood sugar	Serum chlorprop amide mg %	Blood sugar	Serum chlorprop amide mg %	Blood sugar	Serum chlorprop amide mg %
Case 1	2 22	1 66	4 03	1 14	8 21	1 25	6 59
Case 2	1 66	1 53	3 90	1 43	5 30	1 90	9 89
Case 3	3 08	2 00	6 40	1 29	9 23	Not done	Not done
Case 4	2 10	2 67	7 41	1 90	Not done	2 85	6 78

Case 1 Record 492 (E. R.)

Case 2 Record 509 (C. Z.)

Case 3 Record 501 (E. M.)

Case 4 Record 381 (P. V.)

TABLE 7  
TIME OF CHLORPROPAMIDE TREATMENT REQUIRED TO OBTAIN CONTROL OF BLOOD  
SUGAR OR GLYCOSURIA\*

Time of treatment	Blood sugar			Percentage	Glycosuria			Percentage
	No cases		Total		No cases		Total	
	2 gm	1 gm			2 gm	1 gm		
1st Week	9	23	32	58	9	11	31	58.4
2nd Week	5	12	17	31	2	11	13	24.5
3rd Week	2	2	4	7.3	—	1	1	1.9
4th Week	—	—	—	—	1	3	4	7.5
5th Week	1	1	2	3.6	1	3	4	7.5
Total	55 Cases			99.9	53 Cases			99.8

\* Blood sugar below 150 mg per cent; no sugar in 24 hour urine specimen

in response that can be expected due to the different degrees of diabetes, there also may be variations caused by differing rates of absorption and excretion.

*Normalization of blood sugar and disappearance of sugar from the urine.* Blood sugars were returned to normal levels in 55 of the 80 patients. Of these, 58 per cent occurred in the first week (16 per cent in the first 4 days), and 31 per cent in the second week. Disappearance of sugar from the urine occurred at similar times (TABLE 7). There was no evident difference

between those cases receiving 2 gm or those receiving 1 gm during the initial treatment period. Compensation was somewhat more prompt in the hospitalized cases than in the outpatients, possibly because of better observance of the diet.

### Clinical Results

Results are classified as good, fair, and poor. This is the same standard as is used in other publications of this department, with the omission of the category excellent, no curves having been taken for postprandial blood sugar during the day (TABLE 8).

TABLE 8  
STANDARD OF DIABETIC CONTROL\*

Control	Fasting blood sugar mg per cent	24 Hours glycosuria gm	2 Hours postprandial blood sugar mg per cent
Excellent	<150	0	<180
Good	<150	0	<225
Fair	150-200	<20	<250
Poor	>200	>20	>250

\* 180 to 200 gm. of carbohydrates in the diet.

TABLE 9  
RESULTS OF CHLORPROPAMIDE TREATMENT IN 80 PATIENTS

Results	No. Cases	Percentage
Good	44	55.00
Fair	23	28.75
Poor	13	16.25
Total	80	100.00

The results have been analyzed in relation to several factors: the age of the patients, age of onset of the disease, duration of the disease, the patient's nutritive state, complications and associated disorders, previous treatment, especially with insulin, introductory and maintenance doses of the drug, and the serum level of the drug. The results of the selection tests have been compared also with the results of the treatment.

The analysis has not taken into account the differences in reaction between youthful diabetics and middle-aged obese diabetics, these differences are well known, but in practice it is difficult to classify many patients according to rigid categories without doing violence to the facts of the case. In the present analysis greater emphasis has been given to the nutritional state

of the patient a fact that can be determined readily. Results of treatment are presented in TABLE 9.

In practice according to the criterion adopted patients in whom we observed either a good or a fair response were considered subjects for long term treatment with this reservation the fair cases must be subject to rigid observation since it is possible for such cases to develop into failures as time passes even when such development is not due to any factor for example as dietary deficiency or other factors that might affect the

### Age as Related to Response

Treatment failed in the 2 patients under 20 years of age. In the 21 to 40 year group and the group over 40 the percentages of good cases are equal.

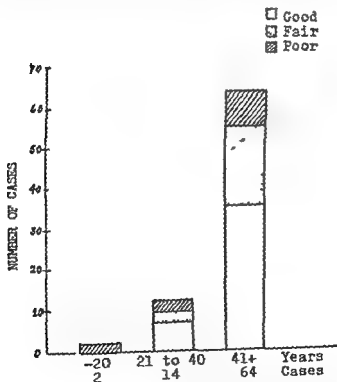


FIGURE 2 Age and results of chlorpropamide treatment in 80 patients.

but the failures are more frequent in the younger patients (21.4 and 12.5 per cent respectively). This tendency reveals a definite correlation of age to response but one that probably would have been more striking had the number of patients involved been greater (FIGURE 2).

*Onset of Diabetes as Related to Result*

This is a complex factor. Aside from considering the special characteristic of diabetes, this factor includes an unknown, the duration of the disease. Nevertheless, both factors suggest the same conclusions: that diabetes in younger patients is habitually refractory to oral treatment and, on the other hand, that there is a possibility that the disease existed for a longer period of time in those who develop diabetes while young (among our patients were 23 more than 40 years old in whom onset of the disease had occurred between the twenty first and fortieth years). It was observed that when the onset of diabetes was between 21 and 40 years of age, the percentage of good results was 47.8 per cent; in those in whom the onset of the disease occurred after the fortieth year, good results were obtained in 60 per cent of the patients.

*Duration of the Disease as Related to Response*

The groups are rather small for a rigorous statistical analysis, but the influence of duration of diabetes seems to be considerable in patients treated with other hypoglycemic drugs.

Of those exhibiting a good result, 89 per cent were patients whose diabetes was of less than one year's duration. Of 22 patients who had had diabetes for more than 10 years, 12 responded well to chlorpropamide, while the remaining 10 showed a fair response. This result may be due to the interference of factors other than the duration of the disease. It may be probable that duration of the disease has little or no direct effect on the result obtained with treatment. It may be of importance in long standing cases in an indirect manner, such as its influence on modifications of the nutritional state that frequently deteriorates in patients not receiving constant treatment.

*Effect of Previous Treatment on Response*

Of the 11 cases that had never been treated with insulin or other measures

of the 13 failures had received insulin previously, and 6 of them also had received treatment with sulfonylureas. However, this result should not be regarded as a direct consequence of the previous treatment factor exclusively, since 90 per cent of the thin, underweight patients had required insulin previously, but only 70 per cent of the obese patients had received it, and the influence that the nutritional state, constitution and type of diabetes exert on the response to oral therapy is well known.

FIGURE 4 shows that the duration of insulin therapy constitutes an adverse factor on the response to treatment with chlorpropamide. In fact, 55 per cent of the patients who had used insulin for less than 5 years had good results with chlorpropamide, and only 25 per cent of the patients who had used insulin for more than 5 years had such results. It should be noted that in the latter group more than half the patients initially had a fair response.

In regard to insulin dosage, even though the patients who receive high

dosage are few we found that in the 7 patients studied who had received more than 40 U daily there was no case in which good results were observed with chlorpropamide therapy. We obtained 4 fair and 3 poor responses in spite of the fact that 4 patients were obese, 2 of normal weight and only 1 was thin. This would confirm the fact that high insulin dosage is an adverse factor. The number of cases studied in our experiments is too small to permit an analysis of the combined effect resulting from the size of the

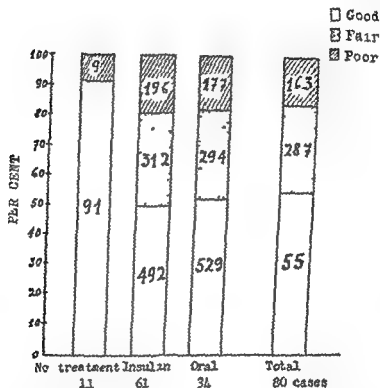


FIGURE 3. Effect of therapy and results of chlorpropamide treatment in 80 patients.

insulin dosage received and the number of years insulin had been administered.

It is interesting to compare results of other types of oral therapy with the results obtained with chlorpropamide. TABLE 10 presents the results obtained using chlorpropamide and other orally applied agents (carbutamide and tolbutamide) on 29 patients. It may be observed that chlorpropamide brought about compensation in 9 of the 14 cases that were failures under the other drugs and achieved good results in 3 of the 6 cases that were merely fair under the other drugs. This is a fact of great therapeutic importance since it offers the possibility of treating patients who have failed to respond to other forms of oral therapy. In TABLE 11 it may be seen that the over all

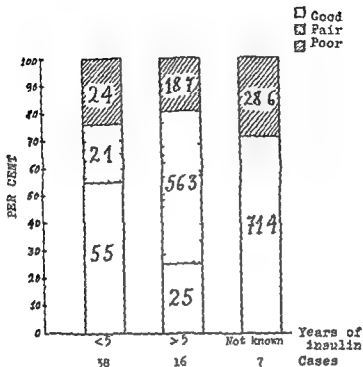


FIGURE 4 Years of insulin and results of chlorpropamide treatment in 61 patients

TABLE 10  
COMPARISON OF CHLORPROPAMIDE TREATMENT WITH OTHER HYPOLYCEMIC AGENTS IN 29 CASES

Chlorpropamide	Other hypoglycemic agents			Chlorpropamide totals
	Good	Fair	Poor	
Good	6	3	5	14
Fair	1	2	4	7
Poor	—	1	3	4
Total	7	6	12	25

TABLE II  
RESULTS OF TREATMENT WITH 3 HYPOGLYCEMIC DRUGS

	Carbutamide		Tolbutamide		Chlorpropamide	
	No. cases	Percentage	No. cases	Percentage	No. cases	Percentage
Success*	169	84	56	72.8	67	83.7
Failure	32	16	21	27.2	11	16.2
Total	201	100	77	100.0	80	100.0

\* Including good and fair results

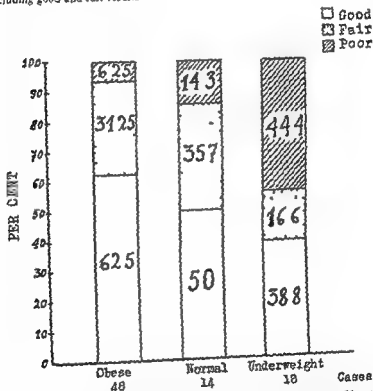


FIGURE 5 Results of chlorpropamide treatment and nutritive status in 80 patients

experience of the authors is that chlorpropamide has demonstrated itself to be as effective as or more effective than carbutamide and tolbutamide in the control of diabetes

#### *Effect of Nutritional Condition on Response*

From FIGURE 5 it is obvious that the highest percentage of success (62.5 per cent) was obtained in obese patients just as is the case with the other sul

fonylureas Even in the group between 20 and 40 years of age the percent  
 the scope of the

As a matter of fact in most of the cases it was impossible since they were generally already receiving a moderate low calory diet to conform with their activity and they still required medication to control their blood sugar levels

### *Effect of Complications or Intercurrent Disorders on Response*

In general the presence of retinopathy nephropathia and tuberculosis or other conditions did not affect the response to an appreciable extent as compared with patients who had no pathology other than diabetes mellitus

that 7 of the 9 tuberculous patients benefited from chlorpropamide

### *Effect of Dosage on Response*

There was no apparent difference in response to an initial dosage of either 2 gm or 1 gm the latter dose obviated in part the appearance of secondary

TABLE 12  
 MAINTENANCE DOSE AND RESULTS OF CHLORPROPAMIDE TREATMENT IN 67 PATIENTS

Result	Maintenance dose in mg					Total
	125	250	500	1000	1500	
Good	2	12	15	13	2	44
Fail	—	—	3	16	4	23
Total	2	12	18	29	6	67
Percentage of Cases	3	18	27	43	9	

reactions especially of a gastrointestinal digestive nature TABLE 12 indicates the maintenance doses employed grouping the patients according to the results It should be noted that among the 13 failures high doses (2 gm daily) were used in 7 cases 1.5 gm doses in 5 cases and 1 gm in only 1 case These were considered failures only after 10 or more days of treatment with these dosages thus ensuring that therapeutic blood levels of the drug had been reached Nevertheless despite the high dosages that often produced intolerance as with the other sulfonylureas poor responses were obtained only in those patients who were not susceptible to oral therapy for various reasons



*Response as Related to Observation Time*

Of the 80 patients studied, treatment was continued in 67 (the 13 failures having been excluded). Of the 67, 62 underwent 30 or more days of treatment.

We record a fact known from treatment with the other sulfonylureas: in some cases, the results change from good to fair or poor as time passes generally within several months. Such patients represent the so called secondary failures. If one eliminates the cases in which decompensation is caused by persistent violations of the prescribed diet or by *intercurrent* disease, one is invariably left with cases for the course of which an explanation is difficult. With chlorpropamide in 15 patients whose treatment was continued from 90 to 210 days, we did not observe this secondary failure phenomenon, however our observation period is still relatively short.

*Response as Related to Serum Level of the Drug*

From TABLE 13 it may be seen that the serum level of the drug varied from 61 to 12 mg per cent in 26 of 34 cases that responded to the drug.

TABLE 13

SERUM LEVELS OF CHLORPROPAMIDE IN PATIENTS RESPONSIVE TO THIS DRUG  
DETERMINATIONS IN 34 PATIENTS

Serum levels of chlorpropamide	Good			Fair			Total
	250	500	1000	500	1000	1500	
31-6	—	2	—	—	1	—	4
61-9	1	5	3	2	1	1	13
91-12	1	2	2	—	7	1	13
121-15	—	—	2	—	—	1	3
211-24	—	—	—	1	—	—	1
Total	2	9	8	3	9	3	34

without any apparent indication that this serum level was related to the response to treatment. Two cases receiving 250 mg of chlorpropamide daily had blood levels that did not differ from those obtained with higher dosage so that this small maintenance dose appeared to be as effective as the higher dosage.

*Comparison of Clinical Results with Earlier Selection Tests*

Response to treatment with sulfonylureas may be predicted to a degree, by studying the hypoglycemic response obtained with a single dose of the

It can be seen from TABLE 14 that of the 40 selection tests in which good results were obtained, only 3 had a poor response to chlorpropamide. However, it was also observed that in 21 patients who showed a poor response to the selection test, only 5 actually showed a poor response to treatment, which renders the test somewhat valueless when it is compared with the clinical indication.

Of greater interest is the test with regard to thin, malnourished patients. It may be observed that in this group the result of the test coincides very

TABLE 14  
SELECTION TEST, NUTRITIVE STATUS, AND RESULTS OF CHLORPROPAMIDE  
TREATMENT IN 67 PATIENTS

Selection test	Obese			Normal			Under weight			Total
	Good	Fair	Poor	Good	Fair	Poor	Good	Fair	Poor	
Good	15	6	2	4	3	1	7	2	—	40
Fair	2	2	—	—	—	—	—	1	1	6
Poor	7	7	1	—	1	—	—	1	4	21
Total	42			9			16			67

well with the response to treatment. The 9 cases with good tests all were susceptible to treatment, of the 5 tests with poor results, in only 1 case was the result of the treatment fair, the other 4 cases being failures. Our experience with thin patients indicates that the test is one that provides a rapid means of selecting those patients susceptible to oral treatment with chlorpropamide.

#### Side Reactions

Side reactions were observed in 24 patients. One third of these patients presented 1 symptom only, one third presented 2 or 3 symptoms simultaneously, and the last third, 4 or more symptoms.

In 15 cases (headache, asthenia, drowsiness, dizziness or vertigo, and muscular pains). The most frequent symptoms were epigastric distress (16 cases) and asthenia (14 cases). The frequency of the other symptoms varied between 2 and 7 cases.

Cutaneous manifestations were mild. In 1 case they disappeared spon-

In the 15 patients who exhibited general symptoms, these were mild in 60 per cent, moderate in 15 per cent, and intense in 25 per cent of the cases.

Side reactions usually appeared between the third and fifth days of treatment (range 1 to 11 days), and lasted 4 to 5 days (range 2 to 14 days). They generally appeared when the patient had received a total of between 5 and 10 gm of the drug (range 1 to 16).

With daily doses of 2 gm we observed reactions in 38.4 per cent of the cases, with 1 gm, in 21 per cent. With a dose of one half gram only, we observed 1 case of pruritus (which disappeared spontaneously) and no other reaction. The reactions were twice as frequent in the patients who responded poorly to treatment. This is understandable, since these patients received much higher doses for a longer period of time in an attempt to control the diabetes. Six of the 13 failures accounted for 31 per cent of the secondary reactions observed.

Since more than 70 per cent of the side reactions were mild or moderate, and since two thirds of those affected presented 1 to 3 symptoms, it was not necessary to suspend treatment in the majority of the cases. The symptoms disappeared spontaneously or, when the dosage was reduced, in 25 per cent of the cases. In only 6 cases was a temporary suspension of treatment necessary, usually for only 2 to 3 days. The treatment was discontinued completely in only 2 cases and, in both of these patients, intolerance to the drug coincided with therapeutic failure. To some patients we administered the drug in wafer form, which suggested (to the patient) a change of medication. This was followed by the rapid disappearance of epigastric distress in patients previously reporting this symptom. This occurrence suggested to us the presence of a psychological component that may have been responsible for the occurrence, intensity, and prolongation of side reactions, especially in neuropathic patients.

du

to . . . . .

It takes the form of a palpitations and, more violent and prolonged 1 to tetraethylthiuram

bisulfide (Antabuse), and can be interpreted as a consequence of blocking the oxidation of alcohol in the acetaldehyde phase.

It is therefore necessary to prevent the patient from consuming any alcohol, even in food. We should note at this point that there were patients who . . . of alcohol (1 gm) at meals without sustaining

recur, so they continued to use a small quantity of wine.

One female patient complained of hypoglycemic symptoms, without laboratory confirmation since they occurred while the patient was at home. On two occasions we noted moderate hypoglycemia in other patients. One of these was a young tuberculous patient, thin, with a diet providing 250 gm of

carbohydrates. He began treatment in intense decompensation which has been controlled exclusively by dietary means.

Finally, one patient without alcoholic, hepatic, or biliary antecedents showed icterus of the cholangiolithic type after 18 days of treatment. Since this case is of especial interest, we reproduce the case history.

Patient aged 44, male, diabetic since 1952, controlled in a very irregular way with protamine zinc insulin and diet, no vesicular or hepatic antecedents. On April 1, 1958, treatment was initiated with 2 gm. daily of chlorpropamide.

TABLE 15  
TESTS

Tests	3/27/58	4/19/58	4/23/58	5/2/58	5/22/58
Chlorpropamide dose	(2 gm. daily 4/1/58 reduced to 250 mg. twice daily 4/11/58 continued to 4/18/58)				
Bilirubin direct 1 min.	0.3	1.8	1.8	0.2	0.3
Total	0.5	3.3	1.4	0.5	1.0
Total cholesterol	208	no	434	220	—
Alkaline phosphatase	12	107	107	107	12
(Manger) flocculation test	(—)	(—)	(—)	(—)	(—)
Prothrombinemia	100%	no	100%	no	—
Bromsulphalein test*	7.6%	no	no	no	0%
Mucoproteins	no	no	4.6	no	no

\* Normal retention less than 6 per cent at 45 min.

pain without radiation and with no correlation to ingestion of food. Also anorexia, dark urine and di-colored stool were noted. On April 15, 1958, conjunctival and cutaneous jaundice appeared associated with moderate pruritus. Prior to treatment with chlorpropamide the patient had shown normal results in the following tests: urine examination, hemogram, sedimentation test, bilirubin determination, alkaline phosphatase test, flocculation test, Bromsulphalein determination, creatinine clearance and phenol-sulfonphthalein test. He was re-hospitalized on April 18, 1958, and chlorpropamide was discontinued.

On physical examination the patient was conscious and lucid, pulse 70, regular, arterial pressure 110/70, no fever, good general condition, moderate icterus of skin and mucosa, hepatomegaly of two fingers' breadth under rib line, spleen not palpable. Other examinations were normal.

The liver went down to normal size two days after admission and the icterus disappeared clinically. He was discharged on May 1, 1958, on insulin daily. Cholecystic

propamide treatment was normal, and the patient has remained in good health up to his last examination (August 23, 1958) flocculation tests, negative, bilirubin determinations normal, and Bromsulphalein retention at 45 minutes 3.6 per cent (normal, less than 6 per cent). We were not able totally to exclude the possibility of a hepatitis of virus origin, frequent in our environment but from the time of appearance, the cholangiolitic character, the brief duration and the subsequent course we are inclined to interpret the case as an icterus of toxic origin (TABLE 15).

#### *Laboratory Determinations*

**Hanger reaction** The hanger reaction was determined in 23 patients. In 21 it was negative and remained so throughout tests made from 20 to 210 days after beginning treatment. In 2 cases it was positive and persisted from the beginning for 27 and 28 days respectively. Suspension of treatment was considered for these two patients.

**Alkaline phosphatase** This enzyme was studied in 21 patients. In 18, it remained normal between 30 and 210 days of treatment; in 2 cases it increased moderately, but became normal at 20 and 28 days, respectively, and it increased considerably in 1 case (cholangiolitic icterus).

**Bromsulphalein retention** Thirty-seven patients were studied in this connection. In 27 cases the results were normal (less than 6 per cent) between days 30 and 210 of treatment. In 3 cases in which the percentages were moderately increased before initiation of treatment, values became normal within 52 and 70 days. Three cases in which Bromsulphalein retention had been increased did not change during a course of treatment lasting 56 to 120 days. In 3 cases that were normal before therapy, very moderate increases occurred in 65 to 107 days (8.3 to 7.8 to 10.4 per cent).

**Prothrombin** This factor was studied in 27 cases. In 25 cases, it remained normal between 30 and 210 days. In 1 case there was a moderate decrease at 84 days (without alterations in other tests). In 1 case there was a decrease that lasted for 7 days before treatment was suspended because of an intervening illness.

**Hemogram** The blood picture was studied in 47 cases. In 3 cases we observed moderate but insignificant decreases in the erythrocytes between 30 and 105 days of treatment. In 1 case there was a very moderate decrease in leukocytes (4200) after 60 days of treatment.

**Urine** Alterations were observed in only one case, where albuminuria (0.64 gm. per cent) appeared after 80 days. The interpretation of this case is difficult. In the 5 cases in which albuminuria had existed previously, there was no modification up to 30 to 120 days of treatment.

#### *Discussion*

The most significant facts encountered in this clinical test of chlorpropamide are the evidence of greater activity of this new sulfonylurea and the increased frequency of secondary reactions when the minimal effective dose is exceeded. Other aspects of clinical response are the same as those observed with the use of similar substances, as may be deduced from analysis of data

in relation to age, nutritional status, and the other variable considerations always taken into account in the oral treatment of diabetes mellitus

The greater activity of chlorpropamide is principally a consequence of the fact that it rapidly reaches effective levels in the blood, probably a result of its being excreted slowly. This also explains the general symptoms observed when doses higher than necessary are used, as found with the first patients we treated. The digestive symptoms, on the other hand, especially the epigastric distress, can be attributed to a definite direct irritating action that probably comes from the presence of chlorine in the molecule. It should be noted that most of these manifestations are only of mild or moderate intensity and of short duration, they usually disappear even with continuation of therapy. From a practical viewpoint, these manifestations do not constitute obstacles to the clinical use of the drug except for an occasional patient who in our experience, usually is also a therapeutic failure.

Examinations of blood and urine and tests of hepatic function revealed no significant modifications in observation periods lasting up to 210 days. The only serious complication might be represented by a case of hepatitis that rapidly ran its course since that time no sequelae have become obvious.

Like the other sulfonylureas, chlorpropamide is active within a few hours

alcohol is ingested during therapy. Apart from the practical interest and importance such a fact has in areas where the consumption of a moderate and permissible amount of alcohol is habitual with meals, there is a theoretical consideration in that it reveals another aspect of the action of the sulfonylureas, one by which they directly modify the intermediate metabolism, acting on the enzymatic processes.

serve only to vary the absolute figures for successful results to a minor degree, at the same time still permitting comparisons to be made among various drugs.

### SUMMARY

Chlorpropamide was administered to 80 diabetics (38 males and 42 females). Two of these patients were less than 20 years old, and 64 were older than 40 years. 48 were obese, 14 of normal weight, and 18 underweight. The diet contained 150 to 250 gm, maintenance dosage ranged from 125 mg to 1.0 gm. Patients were observed from 30 to 210 days. With the initial dose, the immediate hypoglycemic effect was studied on a fasting state. The greatest decrease in the blood sugar level took place at 5 hours in cases that responded (selection test).

Disappearance of glyco-urina and lowered blood sugar levels were obtained in 58 per cent of the cases in the first week and in 31 per cent in the second

week. Results of treatment were good in 55 per cent, fair in 29 per cent, and poor in 16 per cent. In the group exhibiting good results, 75 per cent were observed for more than 60 days, 66 per cent of the cases were maintained on a dosage of 500 mg or less (plasma chlorpropamide ranged between 4.6 and 9.8 mg/100 ml).

Results were correlated directly with nutritional status, age of the patient age at onset of the diabetes, and absence of previous treatment. An inverse correlation was found to duration of treatment and dosage of insulin. No correlation was found to duration of diabetes mellitus.

There were secondary reactions in 24 cases including skin (3), digestive (20), and general manifestations (15). Such symptoms were minor in 72 per cent and disappeared spontaneously after a decrease in dosage or after a short period of discontinuation (6 cases). With a dosage of 1 gm, 21 per cent of reactions were observed as compared with 38.4 per cent when 2 gm was given. One of the patients experienced jaundice of cholangiolitic type after 18 days of therapy. Treatment was discontinued. Alcohol ingestion induced anxiety, palpitations and flushes in 10 cases. No changes were found in liver, kidney, or blood forming functions during or after treatment.

#### ACKNOWLEDGMENTS

We thank M. Usano of Chas. Pfizer & Co., Inc., Brooklyn, N. Y. and Laboratorios Pfizer de Chile, Santiago, Chile for making available the chlorpropamide (Diabinese) used in this study, and for technical data used in determining the presence of chlorpropamide in serum.

#### REFERENCE

1. VALIENTE, S. M. GARCÍA DE LOS RÍOS, I. VIELLA & I. CAÑESSA. Prueba de selección para el tratamiento oral de la diabetes. *Rev. Méd. Chile*. In press.

# CLINICAL STUDY OF THE EFFECTS OF CHLORPROPAMIDE ON NORMAL SUBJECTS AND ON DIABETICS

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Since the year 1955 when Franke and Fuchs published a paper in which they recommended the use of the sulfonylurea carbutamide (1 sulfanilyl 3 butylurea or BZ 55) for the oral treatment of diabetes mellitus there have been numerous articles describing clinical experiments conducted with other compounds including *p* aminobenzenesulfamidyl *tert* butyl (RP 2259) and tolbutamide (1 butyl 3 *p*-tolylsulfonylurea or D 860). At present tolbutamide appears to be preferred by many researchers.

In the hope of finding a product that might be useful to all diabetics and have effective hypoglycemic action and a very low index of toxicity research has continued in that direction. lately there have been tested some derivatives of guanidine especially phenethylformamidineyliminoureia (DBI) and very recently, a new sulfonylurea 1 propyl 3 (*p* chlorobenzenesulfonyl) urea (P 607) commonly referred to as chlorpropamide.

The purpose of this article is to review the results achieved through the use of chlorpropamide (P 607) in persons who showed no disorders of carbohydrate metabolism and in those with diabetes. It was administered to both groups for evaluation of its hypoglycemic activity and for determination of its possible untoward or toxic effects and its therapeutic usefulness.

## PLAN OF STUDY

### *Material and Methods*

Thirty hospitalized patients who evidenced no disorders of carbohydrate metabolism and 60 patients with diabetes mellitus from our private practice

TABLE 1

#### PRIVATE PATIENTS

Total patients studied 60    outpatients 56    inpatients 4  
Sex    male 29    female 31  
Age    average 51 (range 6 to 76)

In tally with other hypoglycemics 20  
Initially with insulin 34  
Patients in more than one group 32  
Subjects of hormone studies 27

the data on whom are compiled in TABLE 1, were selected for this research which covered a period of 90 days. In the course of this work the following studies were made



*Studies in Nondiabetic Subjects Without Disorders of Carbohydrate Metabolism*

With a view to determining the absorption and elimination curve of the compound and the ratio between the blood concentration reached and the hypoglycemic effect obtained the subjects of this study were 30 inpatients (10 males and 20 females) who evidenced no metabolic disorder with respect to carbohydrates and who were divided into 3 similar groups of 10 patients each

After prior stabilization on a prefixed diet (total caloric intake 1833.5 cal carbohydrates 35 per cent 650 cal 162.5 gm, proteins 21 per cent 396 cal 99 gm fats 44 per cent, 787.5 cal 87.5 gm) the subjects were respectively administered a single dose of 0.5 or 1.5 gm chlorpropamide. In all of the patients the blood glucose concentration was determined and in 20 of them that of chlorpropamide in the fasting state and 4 hours later in the manner described by Cameron Dávalos *et al* (1957). The same determinations were repeated in 2 subjects from each group after 8, 12, 48 and 96 hours.

*Studies of Diabetics*

Studies were made of 60 patients classified in 9 different groups with a view to investigating various aspects of the problem: most of them were studied from more than one aspect and therefore appear in more than one group. This accounts for the apparent disparity between the actual number of patients studied and the number resulting from totaling the members of the different groups.

During the research a quantitative diet was indicated for all of the patients the general characteristics of which were as follows: 150 to 200 gm of carbohydrate and 75 to 105 gm of protein supplemented by fats in sufficient quantities to attain the total caloric intake desired for each particular patient.

Described below is the series of 5 biochemical determinations made in addition to the findings of complete clinical examinations conducted with a view to ascertaining the evolution of diabetes and verifying at an early stage any disorder that might occur in the hematopoietic system or in hepatic and renal function: (1) glycemia by Folin and Wu's serum method modified for the determination of true glucose; (2) uremia by Sagastume and Oliva's gasometric method; (3) complete examination of urine; (4) peripheral hemogram and (5) hepatogram including Hanger's test, thymol turbidity, Kunkel's test, alkaline phosphatase, cholesterolemia, bilirubinemia, fractional proteinemia and electrophoresis on paper.

*Group I* This group was composed of 10 patients who initially received a dosage of chlorpropamide for 48 hours of 1.5 gm/day divided into 2 or more doses followed by 1 gm/day for not less than 15 days. For the purpose of verifying the occurrence of untoward symptoms and gastrointestinal tolerance these patients also received at various times and under different circumstances antacid, proportioning of doses and administration concurrently with ingestion of food.

In 5 patients the drug was beneficial and dosage was reduced to a minimum.

effective level. These patients were added to the group of diabetics receiving protracted treatment.

**Group 2** This group included 70 diabetics who were well balanced with other hypoglycemic compounds: 8 received carbutamide and 12 received tolbutamide (TABLE 2). The initial dose of chlorpropamide which was started immediately after suspension of previous therapy was 1 gm/day for 2 days and we attempted to reduce it rapidly to lower levels according to the therapeutic response obtained. The purpose of this procedure was to make a comparative clinical study of various hypoglycemics in relation to such factors as the efficacy of a dose administered for a protracted time.

TABLE 2

Age and sex (present age; females are indicated in parentheses)								
Ages	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80
	—	—	—	—	2 (1)	9 (4)	8 (5)	1 (1)
Age at onset of diabetes								
Ages	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80
	—	—	—	2	8	9	1	—
Years of diabetes at start of treatment								
Years	Recent to 1	1 to 2	3 to 5	6 to 10	11 to 15	16 to 20	21 to 36	
	—	4	5	1	4	3	1	
Body type: pyknic 1; normal 18; long linear 1								
Type of diabetes: stable 20 (100%) ; unstable 0								
Prior drug therapy								
D 860	—			1 gm			1.5 gm	
BZ 55	—			9			3	
	4			4			—	
Duration of prior treatment								
Less than 6 months			6 months to 1 year			1 to 2 years		
4			8			8		
Prior control: good 19; fair 1								

tolerance and the continuance of proper regulation after suspension of the drug.

In all of these patients chlorpropamide therapy was continued for a prolonged period. Five patients from group 1 and 16 from group 4, who also continued to receive the drug, joined them, thereby raising to 41 the total number of subjects in whom the effects of the compound administered for a

**Group 3** This group comprised 9 patients in whom carbutamide and tolbutamide had been ineffective. These patients had received insulin and were selected with a view to drawing clinical conclusions on the similarities between the different compounds. The group includes one case that had presented a good response but that was complicated by skin eruption and icterus.

**Group 4** In this group were 34 cases who had never before been treated with oral hypoglycemics, in 8 of whom no improvement had been produced by dietary restriction, and who had required insulin for years for the proper regulation of their diabetes. The data on the members of this group are set forth in TABLE 3.

When insulin requirements had been lower than 30 U, insulin therapy was suspended, and treatment with an average dose of 1 gm of chlorpropamide was started immediately. On the other hand, in those patients who had received more than 30 U, along with the beginning of treatment with hypoglycemics (125 to 250 mg/day), the dose of which was then gradually augmented, the dosage of insulin was scaled down by a percentage equivalent to 20 per cent of the previous dose every 3 or 4 days.

TABLE 3

Age and sex (present age females are indicated in parentheses)									
Ages	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80	
	3 (3)	3 (1)	1	2 (1)	3 (1)	10 (6)	8 (6)	2 (2)	
Age at onset of diabetes									
Ages	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80	
	5	1	3	4	8	10	2	1	
Years of diabetes at start of treatment									
Years	Recent to 1	1 to 2	3 to 5	6 to 10	11 to 15	16 to 20	21 to 36		
	1	5	12	10	2	3	1		
Body type									
	pyknic 2, normal 28, longilineal 4								
Type of diabetes									
	stable 25 (75 per cent), unstable 9 (25 per cent)								
Prior dose									
	30 U or less					More than 30 U			
		18				16			
Duration of prior treatment									
Less than 1 year		1 to 5	6 to 10	11 to 20		More than 20 years			
		7	11	12		4			
Prior control									
	good 24	fair 10	poor —						

**Group 5** In this group were 6 patients who were well regulated by dietary restriction alone. When under therapy, these patients were permitted a free and ample diet or prescribed 300 gm of carbohydrates daily for the purpose of observing to what extent a sustained dose of chlorpropamide ranging from 125 to 500 mg would make possible an ad libitum diet. They were administered the compound for periods of 30 days or more.

**Group 6** This included 2 infant diabetics and 3 juvenile unstable diabetics, in whom chlorpropamide was tested in doses ranging from 125 mg to 1.5 gm, at the same time we attempted to reduce the usual minimum.

**Group 7** The function by the compounds had already been ascertained.

As part of the determination of serum protein bound iodine (Barker and Humphrey's method), of basal metabolism, and of  $I^{131}$  uptake observed on the gland after 2 and 24 hours, the possible influence that might be exerted by chlorpropamide, when administered over a protracted period in an average dosage of 1 gm daily, was studied.

**Group 8** The following determinations were made in 10 diabetics, with a view to checking any disorder that might occur in hypophyseal and supra-

renal endocrine function hypophyseal gonadotropins by Albright Reifensstein and Griswold's method urinary neutral 17 ketosteroid by Callow, Gallow and Emmen's method and urinary 17 hydroxycorticoids by Reddy Renkins and Thorn's method

*Group III* This was a heterogeneous group of 8 diabetic patients who manifested the special conditions or associated endocrinopathies listed below

These patients were administered chlorpropamide in varying dosages for the purpose of observing the changes that might occur in the existing equilibrium with respect to the carbohydrate metabolism insulin resistance 1

In the last 4 groups control determinations were made whenever it was possible to establish a minimum effective dosage gradually reached after initial doses of 250 to 500 mg

## RESULTS AND THEIR INTERPRETATION

### *Studies of Nondiabetic Subjects*

The results obtained in this study are tabulated in TABLES 4 and 5 in which have been compiled all of the blood glucose concentrations and chlorpropamide values expressed in milligrams per 100 ml of blood

From study of the values transcribed it can be concluded that chlorpropamide when administered orally in humans is rapidly absorbed and reaches efficacious blood concentrations in a few hours The level attained as far as the doses used by us are concerned remained in direct ratio to the quantity administered and was sustained with proportionally the same differences during the first 48 hours After 96 hours there was a marked decline in the values which in 4 of 5 cases observed failed to show any significant differences from one another The decline registered in the glycemic values was quite pronounced within 4 hours after ingestion since the chlorpropamide concentration had by that time reached at least 2.5 to 3.5 mg/100 ml we believe these concentrations are the minimum levels for therapeutic efficacy The hypoglycemic action remained evident for the first 24 hours diminishing during the following hours despite the fact that there were effective blood levels a fact we ascribe to the alimentary ingestion after the first day of observation

It must be emphasized that in this experiment the glycemic decline was always pronounced but did not remain in direct proportion to the blood concentration attained by the drug since the latter had exceeded effective blood levels (greater than 3.5 mg/100 ml)

### *Studies of Diabetics*

*Group I* Seven patients of 10 received high doses for several days and 12 took a high dose only initially Aside from the hypoglycemic effects produced in these patients untoward side effects were manifested (TABLE 6) and on various occasions some of them experienced more than one reaction

TABLE 4

Dose of chlor-prop- amide	Case No	Glucose mg/100 ml		P 607* mg/100 ml 4 hr	Dose of chlor- prop- amide	Case No	Glucose mg 100 ml		P 607 mg 100 ml 4 hr	Dose of chlor- prop- amide	Case No	Glucose mg 100 ml		P 607 mg/100 ml 4 hr
		Fasting	4 hr				Fasting	4 hr				Fasting	4 hr	
0.5 Gm	1	94	73	—	1 Gm	11	92	68	—	1.5 Gm	21	100	70	10.8
	2	78	56	2.8		12	111	62	7.7		22	89	80	—
	3	100	84	1.5		13	83	64	8.4		23	87	77	10.4
	4	92	61	—		14	121	78	—		24	94	72	—
	5	118	69	2.5		15	75	67	—		25	90	60	11
	6	90	70	4.6		16	113	70	10.2		26	120	90	9.4
	7	90	75	—		17	97	—	8.8		27	91	68	10.4
	8	97	68	1.5		18	76	74	8.2		28	77	57	9.6
	9	91	77	—		19	96	72	8.6		29	95	62	10.6
	10	82	69	—		20	103	75	6.7		30	74	55	9.2

\* P 607 = chlorpropamide

TABLE 5

Dose of chlorpropamide	Case No	mg /100 ml	Fasting	4 hr	8 hr	12 hr	24 hr	48 hr	96 hr
0.5 Gm	5	Glucose	118	69	60	70	65	93	92
		Chlorpropamide	—	2.5	5.6	3.3	—	4.8	2.1
	8	Glucose	90	70	93	81	65	90	96
		Chlorpropamide	—	4.6	2.1	3.1	—	2.3	1.5
1 Gm	17	Glucose	97	—	101	72	80	94	108
		Chlorpropamide	—	8.8	5.2	5.7	—	4.8	1.9
	18	Glucose	76	74	75	77	64	101	112
		Chlorpropamide	—	8.2	5.9	5.9	—	5.4	3
1.5 Gm	21	Glucose	100	70	98	88	83	103	100
		Chlorpropamide	—	10.8	12.1	9.0	—	8.6	3.1
	23	Glucose	87	77	47	61	68	90	—
		Chlorpropamide	—	10.4	10.1	10.1	—	7.5	—

TABLE 6

## TOTAL UNDESIRABLE SIDE EFFECTS OBSERVED

Facial flushing and feverishness 9

Skin reactions 4

Digestive disturbances (pyrosis, regurgitation, bloated feeling, anorexia) 10

Constipation, occasional, impossible to determine accurately

Headache 3

Feeling of general malaise with vertigo 2

Increase in blood pressure? 2

It is to be pointed out that reactions of the vasomotor type accompanied by feverishness and facial flushing frequently broke out after ingestion of an alcoholic beverage, spontaneously receding within 30 min and failing to occur when the dose of the drug was reduced or the ingestion of alcohol was suspended.

The skin reactions were largely minor eruptions and on two occasions, became evident after the administration of a saline laxative. In two patients it was necessary to discontinue the drug, application of which could be resumed without side reaction only in smaller doses once the cutaneous episode had subsided.

Gastrointestinal disorders occurring largely in the upper digestive tract were the disturbances most persistently complained of by the patients.

These manifestations could be relieved with the administration of antacids and proportioning of dosage of the drug prescribed and by the ingestion of both at meal times, but the disorders could not be totally eliminated until the dosage was reduced below 1 gm/day

Some patients complained of constipation, occasional headache, and malaise and showed an increase in blood pressure, all of these disturbances were transitory, and their cause and effect relationship with chlorpropamide cannot be defined by the present study. Investigations of liver function and blood count were unsuccessful in relating any of the above mentioned disturbances to a possible attack on the hepatic parenchyma or hematopoietic system. Analyses of blood and urine urea did not show any significant changes.

Five patients in this group subsequently continued therapy with doses of chlorpropamide not exceeding 750 mg/day

*Group 2* In none of the 20 patients who had been kept well regulated with the use of carbutamide or tolbutamide was any disturbance observed when the previous therapy was suspended and chlorpropamide immediately substituted. Chlorpropamide proved efficacious in all of these cases.

tolbutamide the effective dosage of chlorpropamide was determined to be 3 times (in some instances 4 times) smaller than that of the hypoglycemic previously used.

*Group 3* In 4 of 9 patients in whom other oral hypoglycemics had been ineffective the same poor results were observed when chlorpropamide was used. In the other 5 cases chlorpropamide produced satisfactory regulation in contrast to the results previously obtained with carbutamide and tolbutamide. It should be pointed out that in 2 of the latter patients the failure of the drug previously used was due partly to untoward side effects that contraindicated their use (severe icterus). It should also be noted that on 2 occasions patients well balanced with chlorpropamide required insulin during the acute period of an intercurrent febrile process.

*Group 4* In 11 cases receiving an insulin dose of less than 30 U, it was possible to suspend insulin completely and replace it with chlorpropamide. On the other hand only 5 patients receiving more than 30 U of insulin daily could forego the injections.

In 6 cases the insulin dosage, which had ranged between 40 and 70 U, could be significantly reduced (by 20 per cent or more) when chlorpropamide

up this group the addition of chlor  
it possible to broaden the diet to

normal limits without untoward effects. It must be pointed out, however, that repeated dietetic transgressions or excessive gain in weight contraindicated the continuation of this leniency in the majority of the patients.

*Group 6* In none of the 5 members of the group was it possible to suspend insulin entirely; however, in 2 cases it was possible to reduce the dose by

10 per cent of the previous quantity when chlorpropamide was also administered in quantities of 1 and 1.5 gm/day respectively. In a third case the reduction of the insulin dose was equivalent to only 10 per cent of the previous dose but perfect metabolic equilibrium was obtained without hypoglycemia which until then had recurred daily.

**Group 7** In none of the 17 patients making up this group were there any clinical manifestations attributable to hypothyroidism. The variations in values obtained after administration of the drug for long periods and in doses

TABLE 7  
THYROID STUDY

	T <sub>4</sub> (percentages)		Serum protein bound iodine ( $\mu$ g)	Basal metabolism (per cent)	Observations
	2 hr	24 hr			
1	27	47	3.3	+1	Hypothyroidism (under treatment)
2	11	6	1.9	+14	
3	25	47	3.3	+33	
4	20	44	4.1	-3	
5	23	44	3.7	$\pm$ 0	
6	17	36	2.6	+18	
7	20	53	3.6	+8	
8	19	31	4.1	-10	
9				+25	
10				-2	
11	41	62	9.1	+30	Hyperthyroidism
12	33	44		+0	Hyperthyroidism
13	34	56	8.0		
14	10	34	5.1	+40	
15				-12	
16				-9	
17	13	32	3.7	+5	

<sup>a</sup> Resistance in the sense of function.

in a unit.

reaching statistically significant.

a slight tendency to diminish in value (TABLE 7).

**Group 8** The results of the hormonal determinations made in this group of patients and summed up in TABLE 8 do not make it possible to state that chlorpropamide exerts any influence on the glandular functions studied.

**Group 9** Among the 8 patients with special conditions, in the patient who demonstrated resistance to insulin it was possible to reduce the insulin dose from 500 to 60 U/day when chlorpropamide (2 gm/day) was added to the treatment, this drug also contributed to greater metabolic stability.



and relieved the patient of the recurrent periods of chemical acidosis from which he constantly suffered

The 3 patients with allergy to insulin tolerated this new substance without incident. In the patient with the adrenogenital syndrome, in spite of the extraordinarily high content of urinary 17-ketosteroids (120 mg in urine after 24 hours), the response to chlorpropamide was similar to that registered in the cases without endocrinological complications

TABLE 8  
URINARY DETERMINATION OF HYPOPHYSEAL GONADOTROPINS, NEUTRAL  
17 KETOSTEROIDS AND 17 HYDROXYCORTICOSTEROIDS

Case No	Gonadotropins		17 K S mg / 24 hours	17 H K S mg / 24 hours	Observation
	To 6 U L	To 96 U L			
1	(+)	(-)	7.20	2.63	—
2	(+)	(-)	—	—	—
3	(+)*	(-)	6.44	1.68	—
4	(+)	(-)	—	—	—
5	(+)*	(-)	4.68	4.45	—
6	(+)	(-)	5.40	6.98	—
7	(+)	(+)*	1.20	2.19	Adrenogenital syn- drome
8	(+)	(+)	4.62	7.98	Menopause
9	(+)	(+)	8.30	3.41	Menopause
10	(+)	(-)	6.21	4.82	—

\* Weak

In both patients with functional disturbance of the thyroid gland it was extremely difficult to obtain adequate control, as had been the case with all of the treatments previously employed, because of the accentuated metabolic instability with marked ketosis trend constantly present.

In the leprosy patient the response obtained with chlorpropamide was to be expected, taking into account solely his metabolic disorder.

#### COMMENTS

Chlorpropamide is a compound very similar in chemical structure to carbutamide and tolbutamide,



having in common with them the part included in the box drawn with broken lines, but differing in that it has a propyl radical instead of butyl, and that in the para position of the benzene nucleus it has a chlorine instead of the

the absence of the amino radical in chlorpropamide means that it has no bacteriostatic action for which reason undesirable changes in intestinal flora are not registered when it is used. This fact has been corroborated by this study. On the other hand the aforementioned disorders of the upper gastrointestinal tract become common (in about 70 per cent of the subjects studied) when daily doses of more than 1 gm are administered. In no case did we witness such effects when the dosage did not exceed 750 mg/day.

The curves pertaining to nondiabetics reveal the rapid absorption of the compound and its marked therapeutic efficacy in doses of 0.5 gm and even less since after 4 hours the glycemic levels had descended by at least 20 per cent with respect to the initial levels. The greater quantities resulted in a higher blood concentration of the drug and in its slower elimination from the blood stream (good concentrations after 96 hours) which accounts for the protracted therapeutic effect after suspension of the drug and for the high concentrations attained with repeated doses. These observations coincide with other experimental and clinical findings (Goldner 1958) to the effect that effective blood levels attained after 48 hours slowly drop on suspension of the drug until its complete elimination from the blood stream within a period of 5 to 7 days. According to Heinzen (1959) 50 per cent of an oral dose will still remain in the blood after 24 hours. This opinion is shared by Miller (1958) according to whose experience with a dose of 1 gm/day the blood concentration rises gradually for a period of 1 week and is then maintained at the last levels reached.

In one of our cases in which the maintenance dose was 1.5 gm/day 25 days after treatment the blood chlorpropamide concentration was 16.5 mg/100 ml. In 3 other patients receiving 750 mg over the same period the values were 8.4, 10.8 and 13.3 mg/100 ml respectively. In one of the patients who received 500 mg/day the figure was 9.4 mg/ml and on the other hand in 2 other cases with the same dose the values were 4.6 mg and 5.4 mg/100 ml which reveals the importance of the size of the dose by itself and the variations that can arise according to the individual susceptibility of each patient.

Taking the foregoing into account and also the fact that the hypoglycemic effect bore no direct relationship to the dosage since effective therapeutic levels had been attained it does not seem hazardous to affirm that the quantities indicated for clinical use should not exceed 750 mg/day whether in single or proportioned doses the more so since the risks of hypoglycemia and the onset of untoward side effects become more common with higher doses.

Without being able to point to a definite pathogenic mechanism (perhaps the inhibition of alcoholic dehydrogenation) mention should be made of the frequency with which we observed a reduced alcoholic tolerance manifested by feverishness and facial flushing, which ceased with the reduction of the dose or suspension of alcohol.

The possibility of satisfactorily regulating diabetics who had previously been receiving carbutamide or tolbutamide successfully was confirmed in 100 per cent of the cases studied in such connection and it must be added that chlorpropamide was also efficacious in cases that had not responded to

other drugs. This therapeutic efficacy becomes evident with doses that are usually 3 or 4 times smaller than those of carbutamide and tolbutamide.

In almost all of our case histories the effective quantity of chlorpropamide for prolonged treatment was less than 1 gm./day, in many instances only 0.5 gm., and in several cases 0.25 gm. and 0.125 gm./day were efficacious. This fact bears out the observations made by Root (1958) in the rat, when she showed that chlorpropamide was 20 per cent more active in this animal than carbutamide and 60 per cent more efficacious than tolbutamide.

In all of our cases the blood chlorpropamide concentration was maintained at effective levels and, when the doses were 1 gm. or larger, the figures rose significantly, as already mentioned with reference to the results. In no instance was any definite attack on the hepatic or renal parenchyma or hematopoietic system evidenced although, on two occasions, positive liver flocculation tests and the elevation of alkaline phosphatase observed in patients maintained for a relatively long time on 1 gm. of the compound raised a question as to the harmlessness of the substance to the parenchyma of the liver when administered over a protracted period of time in high dosage (see results among 15 patients in TABLE 9).

In general the good therapeutic responses in our patients maintained the same relationship with various factors as those reported by Menhert *et al* (1958) with respect to tolbutamide: the onset of disease in middle age, small previous insulin requirements, short history of diabetes, and recent insulin treatment.

The foregoing bears out the therapeutic inefficacy observed in infantile and juvenile patients and in several of those patients who required insulin doses of more than 30 U, in all of whom the continuation of treatment with both drugs seems indicated only when necessary to attain metabolic stability that cannot be achieved by other means, since not only have we been able to observe a diminution of broad glycaemic variations, but also a marked "warding off" of ketosis, which in several cases was readily manifested when insulin alone was used.

In our cases the reduction of insulin dosage among patients who had depended exclusively on this hormone for regulation ranged between 10 and 100 per cent. This bears out similar observations made in humans with respect to tolbutamide by Minsky and Diengott (1957) and by Fabrykant (1958), which confirm those carried out in the rat with tolbutamide by Houssay *et al* (1957) and with chlorpropamide by Root (1958).

The increased metabolic stability offered by some oral hypoglycemics has also been pointed out with special reference to carbutamide by Izzo (1957), to tolbutamide by Fulner *et al* (1956), Bander (1956), Fabrykant (1958), and to various biguanidines by Krall and White (1958).

It is interesting to note that several patients who were controlled previously with both insulin therapy and later with chlorpropamide claimed that they felt better with the latter substance. This fact was more pronounced in children, even in periods of unsatisfactory compensation.

This feeling of good health made it necessary to be extremely vigilant as to observance of the prescribed diet, for patients might easily commit serious

Table 9

Case No	Hanger test	Thymol turbidity	Runkel test	Alkaline phosphatase	Cholesterol	Bilirubin	Proteins	Electrophoresis	Hemogram
1	(-)	2.1	5.2	3	262	D = 0.05 I = 0.55 Tot = 0.6	$\lambda$ = 4.46 G = 3.25 Tot = 6.69 $\lambda$ G = 2	$\lambda$ = 3.01 = 45% $\alpha_1$ = 0.27 = 4.1% $\alpha_2$ = 0.80 = 12% $\beta_1$ = 0.80 = 12.1% $\beta_2$ = 0.35 = 5.3% $\gamma$ = 1.43 = 21.5%	
2	(-)	0.5	2.5	2.64	260	D = 0.1 I = 0.5 Tot = 0.6	$\lambda$ = 4.62 G = 2.61 Tot = 7.23 $\lambda$ G = 1.76	$\lambda$ = 3.75 = 51.9% $\alpha_1$ = 0.45 = 6.3% $\alpha_2$ = 0.74 = 10.3% $\beta$ = 0.93 = 13% $\gamma$ = 1.31 = 18.2%	
3	(-)	4	6	16.4	202	D = 0 I = 0.4 Tot = 0.4	$\lambda$ = 4 G = 2.46 Tot = 6.46 $\lambda$ G = 1.62	$\lambda$ = 3.30 = 51.2% $\alpha_1$ = 0.27 = 4.3% $\alpha_2$ = 0.73 = 11.4% $\beta$ = 0.74 = 11.5% $\gamma$ = 1.36 = 21.4%	
4	(-)	3.5	6	2.68	250	D = 0.1 I = 0.6 Tot = 0.7	$\lambda$ = 4.46 G = 2.54 Tot = 7 $\lambda$ G = 1.71	$\lambda$ = 3.74 = 53.4% $\alpha_1$ = 0.29 = 4.12% $\alpha_2$ = 0.71 = 10.12% $\beta$ = 0.66 = 9.43% $\gamma$ = 1.60 = 22.85%	

TABLE 9 (Continued)

Case No	Hanger test	Thy mol turbidity	Kunkel test	Alkaline phosphatase	Cholesterol	Bilirubin	Proteins	Electrophoresis	Hemogram
5	(-)	2	4.5	6.18	242	D = 0.15 I = 0.65 Tot = 0.80	A = 4.31 G = 2.77 Tot = 7.08 A/G = 1.55	$\lambda$ = 3.49 = 49.3% $\mu$ = 0.22 = 3.2% $\alpha_1$ = 0.62 = 8.8% $\beta$ = 1.17 = 16.6% $\gamma$ = 1.55 = 21.9%	
6	(-)	3.1	5.8	10.1	216	D = 0 I = 0.5 Tot = 0.5	A = 4.64 G = 2.90 Tot = 7.54 A/G = 1.60	$\lambda$ = 3.55 = 47.2% $\alpha$ = 0.23 = 3.1% $\alpha_1$ = 1.17 = 15.6% $\beta$ = 0.98 = 13% $\gamma$ = 1.57 = 20.9%	
7	(-)	4.5	10	3.5	230	D = 0.15 I = 0.70 Tot = 0.85	Tot = 6.55		RBC 400,000 WBC 5,900 Hb 98%
8	(-)	5	6	1.46	290	D = 0.1 I = 0.58 Tot = 0.68			RBC 5,100,000 WBC 6,900 Hb 92% Diff $\gamma$ = 59% I = 7 B = 0 L = 30 M = 4
9	(+)	4.2	5	2.33	260	D = 0.05 I = 0.61 Tot = 0.66			RBC 4,640,000 WBC 5,300 Hb 76% Diff N = 62% I = 4 B = 0 L = 30 M = 2

30	(++)	2.02	4.5	5.38	2.30	D = 0.05 I = 0.50 Tot = 0.55			RBC 5 080 000 WBC 6 300 Hb 96% V G = 0.94 Diff N = 72% I = 3 B = 0.1 = 22 V = 3
11	(++)	3.8	4.2	2.02	315	D = 0.1 I = 0.68 Tot = 0.78			RBC 5 050 000 WBC 10 000 Hb 14.5 G Diff N = 61% I = 1 B = 0.1 = 33, V = 4
12	(+++)	17.7	2.3	8.49	1.95	D = 0.15 I = 0.75 Tot = 0.9			
13	(+++)	5.6	8.5	5.32	206	D = 0.1 I = 0.59 Tot = 0.69			RBC 5 580 000 WBC 8 100 Hb 16.2 G = 108% V G 0.93 Diff N = 62% L = 0 B = 0.1 = 34 V = 5
14	(-)	1.5	2	3	212	D = 0 I = 0.6 Tot = 0.6			RBC 4 900 000 WBC 7 000 Hb 12.8 G = 86% Diff N = 69% E = 2, B = 0.1 = 26, V = 3
15	(+)	5.5	7.2	3.49	262	D = 0.65 I = 0.64 Tot = 0.69			RBC 4 700 000 WBC 6 400 Hb 12.6 G = 84% Diff N = 67% I = 1 B = 0.1 = 25 V = 4

# BLOOD LEVELS OF CHLORPROPAMIDE IN NORMAL MEN FOLLOWING CHRONIC ADMINISTRATION

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As part of a large study program on the efficacy and activity of the new oral hypoglycemic agent chlorpropamide, it was felt that a knowledge of the blood levels at various dosages following chronic oral administration would be of importance. We were already aware that only small amounts are excreted in the urine. Therefore it seemed necessary to determine whether serum levels continued to rise with repeated administration or whether some plateau value would ensue.

## Method

Three groups of normal male volunteers were placed on a daily dosage schedule of either 0.25, 0.50 or 1.0 gm chlorpropamide daily. The group

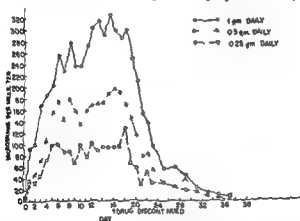


FIGURE 1 Chlorpropamide serum levels

taking the highest dose was composed of 20 men, the other 2 groups comprised 10 men each. A fasting blood sample was drawn on the morning of the first day and the drug was administered orally immediately afterwards.

received 100 mg daily. The entire group on 1.0 gm daily had the drug discontinued at the end of 15 days, and the serum levels were followed through the thirty-seventh day. Usual diets were maintained throughout the study period except for added carbohydrates as needed to relieve sensations of weakness and hunger. Analyses of the serum for chlorpropamide were carried out in our laboratories by the method of Toolan and Wagner.<sup>1</sup>

TABLE 1  
BLOOD LEVEL OF CHLOROPROMIDE 0.25 Gm Daily ( $\mu\text{g/ml}$ )

Patient	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	27	29	31	33	35	37
21	63	30	41	43	95	87	35	43	35	77	43	98	77	40	63	80	75	48	47	18	37	10	0	35	42	0	0	0	0	0	0
22	44	30	40	83	75	90	75	—	—	—	—	—	—	—	48	75	82	250	55	61	25	48	32	—	—	15	30	—	10	10	10
23*	55	41	47	78	125	135	120	140	85	112	103	185	138	110	160	118	207	130	125	122	90	110	110	15	77	50	40	32	25	20	20
24	45	40	45	18	98	103	77	80	60	75	58	100	75	85	85	93	113	—	—	—	—	—	—	—	—	—	—	—	—	—	—
25*	43	42	76	74	113	95	88	130	100	162	92	125	90	123	100	120	110	123	110	125	90	125	50	40	50	40	35	12	25	20	—
26*	46	52	42	67	110	115	127	118	70	145	98	107	93	113	105	123	123	105	102	85	70	82	68	—	50	55	45	55	—	25	—
27	39	43	41	73	92	110	83	92	70	85	65	50	80	90	97	98	83	82	60	47	30	50	30	85	15	10	18	20	—	10	—
28	39	44	08	97	117	103	90	120	40	85	90	100	90	140	123	125	125	107	102	72	38	67	48	10	18	10	—	12	0	0	0
29*	40	44	69	62	50	87	75	77	60	80	75	75	60	87	102	114	128	85	55	70	58	70	57	48	42	15	30	20	10	10	10
30*	43	40	64	73	85	92	75	100	77	77	75	70	65	98	68	102	85	70	48	18	62	47	—	15	40	0	0	0	0	0	0
%mean	46	42	44	73	98	100	86	88	66	99	78	101	88	95	95	94	96	129	66	57	28	51	30	32	23	19	16	11	3	5	5
														95	95	119	131	102	92	90	65	90	60	34	47	42	30	28	18	15	15

\* Started on 100 mg daily on sixteenth day others discontinued



TABLE 2  
BLOOD LEVEL OF CHLOROPROPANIDE, 0.5 G<sup>m</sup> DAILY ( $\mu\text{g}/\text{ml}$ )

Patient	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	27	29	31	33	35	37	
31	15	24	48	63	93	90	85	119	98	110	95	115	110	130	150	123	115	90	90	50	30	25	0	0	0	—	0	0	0	0	
32*	33	62	85	156	113	197	177	235	188	238	235	230	240	263	250	270	293	410	222	162	132	182	155	27	80	67	42	—	0	35	25
33*	30	63	90	122	151	190	173	220	160	185	183	200	200	195	220	250	220	180	180	122	90	125	105	62	50	35	35	—	25	20	20
34*	21	48	80	98	190	115	108	160	165	90	148	100	162	180	185	175	167	150	135	105	88	—	65	55	33	20	12	0	0	0	0
35	20	46	63	123	163	165	142	190	145	130	145	198	200	190	227	220	200	190	165	90	80	75	57	47	10	22	20	0	0	0	0
36	34	57	109	153	230	265	188	245	212	215	233	250	253	260	283	275	233	200	190	150	85	125	77	63	30	32	20	30	0	0	0
37	36	65	110	110	210	190	150	230	200	160	210	223	165	235	193	193	—	220	177	170	115	120	90	—	50	30	20	35	20	10	0
38*	31	49	65	84	123	125	100	127	120	112	110	105	110	—	140	145	123	112	80	90	50	75	57	42	—	50	20	50	20	15	0
39	30	52	78	115	226	250	157	212	240	177	170	192	205	225	187	177	220	150	115	125	90	77	57	10	30	18	—	0	0	0	0
40†	5	0	0	36	60	—	—	63	58	33	75	65	52	80	55	120	83	50	30	30	0	30	—	0	0	0	0	0	0	0	0
															Mean off drug	198	192	170	147	117	80	111	56	30	25	18	17	13	4	2	
Mean	26	47	73	106	156	176	142	180	159	135	160	168	170	173	189	210	201	213	154	127	90	127	96	47	54	43	27	25	20	15	

\* Started on 100 mg daily on nineteenth day, others discontinued.

\* Started on 100 mg daily on sixteenth day, others discontinued

† Excluded from mean

Table 3

Patient	Day 1	25	27	29	31	33	35	37
1	105	30	35	40	18	—	0	0
2	85	93	62	72	52	27	15	10
3	92	40	15	15	10	0	1	0
4	85	50	45	50	30	35	10	10
5	91	40	18	25	15	0	0	0
6	100	68	70	62	35	27	20	10
7	128	—	40	60	10	20	10	10
8	88	35	30	25	40	0	10	0
9	93	50	40	50	10	20	10	10
10	79	38	40	42	15	12	0	0
11	75	90	50	48	35	40	20	12
12	82	63	47	42	18	0	0	0
13*	111	0	40	48	25	0	0	0
14*	0	12	12	30	17	10	0	0
15	77	60	65	52	15	0	0	0
16	80	65	62	35	12	0	0	0
17*	61	20	35	60	18	0	0	0
18	98	73	77	50	27	—	10	10
19	0	77	153	80	35	50	20	20
20	77	18	—	0	0	0	0	0
Mean	92	57	39	45	24	14	9	5

\* Excluded from mean, a

*Results and Discussion*

The average daily serum levels of chlorpropamide for the three dosages administered are given in FIGURE 1. TABLES 1, 2, and 3 show that the individual serum levels within each group were spread over a considerable range

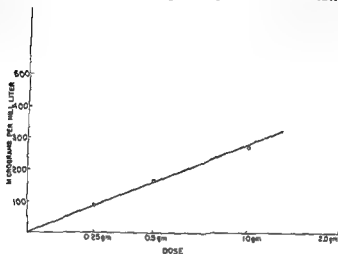


FIGURE 2 Log dose chlorpropamide serum level curve

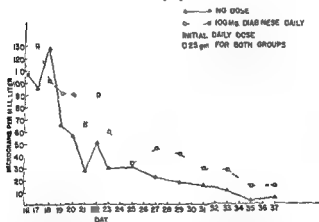


FIGURE 3 Effect of chlorpropamide—100 mg daily—on maintenance of serum level of values. However, each subject, on a day-to-day basis, had reasonably consistent values. It is apparent that at 0.25 gm and at 0.50 gm daily the serum levels reach a plateau in 3 to 5 days around which there is some daily variation. At 1.0 gm daily it appears that the plateau is reached somewhat

siderably slower than that for tolbutamide and presumably, accounts for the more prolonged effect that has been reported. FIGURES 3 and 4 demon-

were maintained on chlorpropamide at 100 mg daily after discontinuing the prior larger daily doses as in those where the medication was discontinued

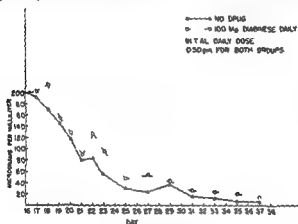


FIGURE 4

after 15 days. During the course of the study there were no subjective or objective symptoms observed. However many of the subjects did experience hunger and supplemented their diet with chocolate and hard candies.

#### Summary

Various doses of chlorpropamide were given orally to normal volunteers. Serum levels were followed for thirty seven days. No untoward effects were observed.

#### References

1. TOOLAN T & R I WAGNER JR 1959 The physical properties of chlorpropamide and its determination in human serum. *Ann N Y Acad Sci* 74(1) 449

## SULFONYLUREA DRUGS IN THE TREATMENT OF DIABETES MELLITUS

P B Landabure, C Delbue, C Alvarinas, N Serantes,  
R Camerini Dávalos, Gregorio Waisman, A Cabarro, E Dussel,  
F Cicchitti, A Loreda

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### INTRODUCTION

The National Institute of Nutrition of Argentina first began clinical investigation of the sulfonylurea drugs with carbutamide in March 1956. The results were reported at a meeting of the Argentine Association of Nutrition and Dietetics in October 1956 and were published in 1957.<sup>1</sup> We expanded our work to include tolbutamide, and a report of the results obtained with this drug was also published.<sup>2</sup> Some of us have reported separately on our experiences with the sulfonylurea drugs.<sup>3-11</sup> Two conferences were held on sulfonylurea drugs by the Argentine Medical Association (in 1957 and 1958) and two special sessions were held by the Argentine Association of Nutrition and Dietetics.

Theoretically, the ideal sulfonylurea drug would provide the following (1) minimal gastrointestinal intolerances, with no acute or chronic side reactions or toxicity and an ample margin of safety, that is, a wide difference between the therapeutic dose and the toxic dose, (2) a favorable action in  
-nutritional  
recurrent  
al physio

logical mechanism that maintains sugar metabolism

An analysis of all available preparations reported in the literature was published by one of us in June 1958<sup>6</sup> and is summarized in TABLE 1. It is evident from the ample literature of the past three years that we are far from achieving the ideal therapeutic agent. For this reason, the appearance of any hypoglycemic drug makes it mandatory that we determine how close it comes to the therapeutic ideal and establish a comparison with insulin and drugs previously evaluated in regard to efficacy, dosage, intolerance, and possible future effect on the therapy of diabetes.

With this point of view in mind the National Institute of Nutrition, the Chair of Pathology, and the Nutrition Clinic, in association with other Argentine centers for the study of diabetes situated in La Plata, Mendoza, and Córdoba carried out a study of the clinical effects of chlorpropamide. The method involved rigid conformance to the same techniques at different places, which invests this project with both a unity and a diversity that affect the significance of the results.

TABLE 1  
CONSENSUS ON ACTION OF AND INDICATIONS FOR BENZENESULFONAMIDES\*

Benzenesulfonamides	Probable locus or mode of action
<p><i>p</i>-aminobenzenesulfamidoisopropylthiodiazole</p>	(1) Pancreas
<p><i>p</i>-aminobenzenesulfonylbutylurea</p>	(2) Inhibition of insulin degradation (3) Liver enzyme systems
<p><i>p</i>-methylbenzenesulfonylbutylurea</p>	(1) Peripheral effect on carbohydrate metabolism
Criteria of case selection	Factors indicating use of benzenesulfonamides
(1) Age (2) Duration of diabetes (3) Duration of insulin treatment (4) Insulin dosage (5) Constitutional type (6) Fasting blood sugar level before treatment (7) Ketosis tendency	(1) More than 50 years old (2) Less than 10 years (3) Less than 5 years (4) Less than 40 U (5) Pyknic, with obesity and essential hypertension tendencies (6) Not more than 300 mg /cc (7) None

\* To June 1958

## MATERIAL AND METHODS EMPLOYED

### *Characteristics of the Patients Studied*

The drug was used on 200 patients, but data have been tabulated for only 133; these patients have been treated for a sufficient length of time to permit an evaluation to be made (FIGURE 1).

### *Conditions Under Which the Study Was Conducted*

*Form of control* Of the total of 130 patients whose data have been tabulated, 30 (23 per cent) were studied while they were hospitalized, and 100 (77 per cent) were observed in the outpatient department. The latter were

selected from patients who assured us that they would follow instruction carefully. To evaluate fully the effect of the drug we administered it to patients having various types of diabetes. After observations of response were made, we continued our investigation of those individuals who had some degree of satisfactory response to the drug.

*The diet followed.* A nutritional study was conducted in order to acquire a knowledge of the diet of all patients. A dietary interview was held with each patient during which a detailed history of his dietary habits was recorded.

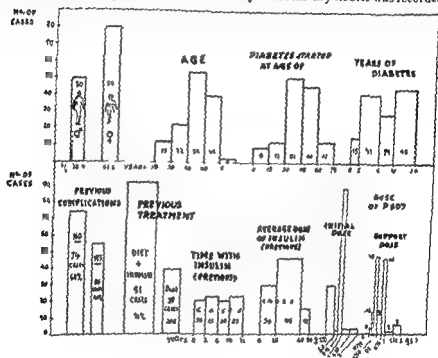


FIGURE 1. Characteristics of 130 cases under observation

Dietary regimens, with the characteristics of these normal regimens introduced, were designed to provide each patient with the caloric value appropriate for his or her nutritional status, and to include approximately 200 gm of carbohydrates, this amount was reduced in certain cases and increased in others to as much as 300 gm. It is understood, of course, that the other values—those of the minerals and vitamins—were the same as in the normal regimen in every case.

*Examinations and tests used to evaluate the response to therapy.* The observation of each patient in the study was carried out under strict clinical, medical, and laboratory control. Clinical determinations included body weight, diuresis, and any subjective or objective symptoms that appeared. Laboratory determinations included blood sugar before breakfast, blood sugar profile during the day, total daily urine sugar and urine sugar profile, exist-

ence of acetonemia, complete urinalysis, protidemia, lipemia, urea in the blood, liver function tests, hemogram, renal adequacy test and, in a sizable number of cases, liver biopsy puncture<sup>12</sup> In the hospitalized patients, a study of the carbohydrate metabolism was performed with the standard curves (Allbright's technique), intravenous glucose, intravenous insulin glucose and adrenalin insulin In six cases the action of chlorpropamide in intravenous administration was studied

#### *Dosage Schedule for Chlorpropamide*

The drug was administered in most of the cases in 2 doses (1 after breakfast and 1 after lunch) In some patients the drug was given after dinner The highest total dosage administered thus far is 71 gm, the longest duration of therapy 130 days All patients are continuing on their respective dosage schedules

#### *Techniques and Standards Used*

Addis counts were made by the usual technique Blood tests were made as follows urea, modified enzymatic technique of Wrenn, glucose, the

TABLE 2

DEFINITIONS OF STANDARDS OF CONTROL AND EVALUATION FOR CHLORPROPAMIDE

Control	Blood sugar mg % (Somogyi Nelson)	Total urine sugar gm	Acetone
Excellent	80-110	none	none
Good	111-130	To 5 per cent of the food glucides	none
Fair	131-170	To 10-20 per cent of the food glucides	none
No control	Higher levels	Higher levels	—
Efficient			
Partially efficient			
Inefficient			

Somogyi Nelson technique alkaline phosphatase, the Bodansky method blood proteins the techniques of Calvary, Cohn, Wolfson and Ichiva cholesterol, the method of Bloor, Sheftel, and Hill, MacLagan, the original technique with adaptation of electrophotometric reading, Hanger, the

#### *test, the usual technique*

Designations for standards of effectiveness of chlorpropamide in the control of diabetes in relation to blood sugar and glycosuria are shown in TABLE 2 Diabetic groupings according to response to chlorpropamide are shown in TABLE 3



# BLOOD LEVELS OF CHLORPROPAMIDE IN NORMAL MEN FOLLOWING CHRONIC ADMINISTRATION

Michael Carlozzi, Domenic G Iezzoni, Lawrence Silver  
Chas Pfizer & Co Inc Brooklyn N Y

As part of a large study program on the efficacy and activity of the new oral hypoglycemic agent chlorpropamide it was felt that a knowledge of the blood levels at various dosages following chronic oral administration would be of importance. We were already aware that only small amounts are excreted in the urine. Therefore it seemed necessary to determine whether serum levels continued to rise with repeated administration or whether some plateau value would ensue.

## Method

Three groups of normal male volunteers were placed on a daily dosage schedule of either 0.25, 0.50 or 1.0 gm chlorpropamide daily. The group

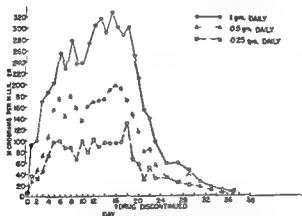


FIGURE 1 Chlorpropamide serum levels

taking the highest dose was composed of 20 men, the other 2 groups comprised 10 men each. A fasting blood sample was drawn on the morning of the first day and the drug was administered orally immediately afterwards.

received 100 mg daily. The entire group on 1.0 gm daily had the drug

ried out in our laboratories by the method of Toolan and Wagner<sup>1</sup>

TABLE I  
BLOOD LEVEL OF CHLOROPROPAMIDE, 0.25 C = 10 μg (μg/ml)

Patient	12a	1	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	27	29	31	33	35	37
21	63	30	43	43	95	8	35	43	35	77	43	90	7	40	61	80	5	75	48	6	18	37	10	0	35	42	0	0	0	0	0
22	44	30	40	31	—	73	90	375	—	—	—	—	—	50	48	75	82	250	55	61	25	48	32	—	—	—	15	30	—	10	10
23*	55	41	4	8	125	145	120	130	85	112	101	185	158	110	160	196	207	130	125	121	90	110	80	13	77	40	40	32	25	30	20
24	35	49	43	8	99	100	77	80	60	75	58	100	51	—	55	93	113	—	—	—	—	—	—	—	—	—	—	—	—	—	—
25*	43	42	6	74	113	95	88	30	100	167	92	121	109	123	100	120	110	123	110	125	90	125	50	40	50	50	35	32	25	20	20
26*	46	52	67	110	113	127	118	70	135	98	107	93	111	105	105	123	123	110	102	85	70	82	68	—	50	55	45	35	—	25	25
27	39	61	41	3	92	110	81	92	70	85	65	50	80	90	97	94	83	82	60	47	30	50	30	85	15	10	18	20	—	10	10
28	39	44	68	97	111	103	90	120	40	55	90	100	90	140	123	123	125	107	102	72	38	67	48	10	18	10	—	12	0	0	0
29*	40	45	69	62	80	87	5	77	60	80	75	75	60	87	102	113	128	81	55	70	58	0	57	48	42	15	30	20	10	10	10
30*	43	40	64	73	85	92	75	100	77	77	75	6	65	98	68	102	85	0	20	48	18	62	47	—	15	40	0	0	0	0	0
Mean	46	42	54	73	98	100	86	88	66	93	8	101	88	95	95	119	131	102	97	90	65	50	60	34	47	42	30	28	33	15	15
														Mean no drug		94	91	129	66	57	28	51	30	12	25	19	16	11	5	5	5

\* Started on 100 mg daily on sixteenth day others discontinued

TABLE 2  
BLOOD LEVEL OF CHLOROPANIDE OF C<sub>10</sub> DAILY (µg ml<sup>-1</sup>)

Latent	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	
31	15	23	48	64	94	90	85	119	98	110	98	114	110	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130	130
12*	15	64	83	130	135	131	177	133	183	238	238	238	240	263	263	263	263	263	263	263	263	263	263	263	263	263	263	263	263	263	263	263	263	263	263	263	263	263
21*	30	63	90	123	151	190	173	230	160	165	181	200	209	195	195	195	195	195	195	195	195	195	195	195	195	195	195	195	195	195	195	195	195	195	195	195	195	195
33*	21	58	80	98	100	115	104	160	163	90	148	160	162	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180	180
35	20	46	65	12	164	165	147	190	145	130	145	198	200	190	227	227	227	227	227	227	227	227	227	227	227	227	227	227	227	227	227	227	227	227	227	227	227	
36	26	57	109	131	236	265	185	245	212	215	233	240	253	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	
37	26	65	110	110	210	190	140	230	200	160	210	233	165	235	235	235	235	235	235	235	235	235	235	235	235	235	235	235	235	235	235	235	235	235	235	235	235	
38*	35	49	65	85	123	125	100	137	110	133	130	103	130	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
39	30	52	78	115	226	240	157	212	240	177	110	192	203	225	225	225	225	225	225	225	225	225	225	225	225	225	225	225	225	225	225	225	225	225	225	225	225	
40†	5	0	0	36	60	—	—	53	50	33	75	65	52	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Mean	26	49	73	106	156	176	142	180	150	135	160	168	170	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	
Mean	26	49	73	106	156	176	142	180	150	135	160	168	170	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	173	

\* Started on 100 mg daily on seventh day, others discontinued

† Actual from mean

TABLE 3  
BLOOD LEVEL OF CHLOROPROPANIDE 10 Gm DAILY ( $\mu$ g/ml)

Patient	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	27	29	31	33	35	37
1	105																							30	35	30	18	—	0	0
2	85																							93	62	72	32	27	15	10
3	92																							40	15	35	10	0	0	0
4	82																							50	45	50	30	35	10	10
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6	100																							68	70	62	35	27	20	10
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8	88																							35	30	25	40	0	10	0
9	93																							50	50	50	30	20	10	10
10	79																							38	40	42	15	12	0	0
11	75																							90	50	48	45	40	20	12
12	82																							65	47	42	18	0	0	0
13*	115																							0	40	48	25	0	0	0
14*	0																							12	12	30	17	10	0	0
15	77																							60	65	52	15	0	0	0
16	80																							65	62	35	12	0	0	0
17*	63																							20	35	60	18	0	0	0
18	98																							73	77	50	27	—	10	10
19	0																							77	153	80	35	30	20	20
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*Results and Discussion*

The average daily serum levels of chlorpropamide for the three dosages administered are given in FIGURE 1. TABLES 1, 2, and 3 show that the individual serum levels within each group were spread over a considerable range

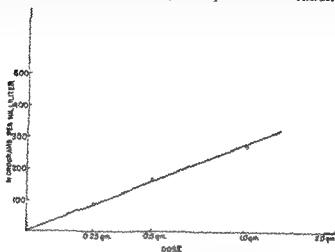


FIGURE 2 Log dose chlorpropamide serum level curve

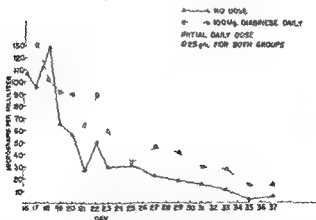


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siderably slower than that for tolbutamide and presumably accounts for

chlorpropamide levels is substantially the same in those individuals who were maintained on chlorpropamide at 100 mg daily after discontinuing the prior larger daily doses as in those where the medication was discontinued

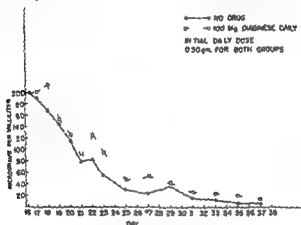


FIGURE 4

after 15 days. During the course of the study there were no subjective or objective symptoms observed. However many of the subjects did experience hunger and supplemented their diet with chocolate and hard candies.

#### Summary

Various doses of chlorpropamide were given orally to normal volunteers. Serum levels were followed for thirty seven days. No untoward effects were observed.

#### Reference

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## INTRODUCTION

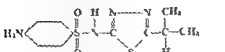
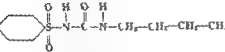
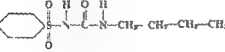
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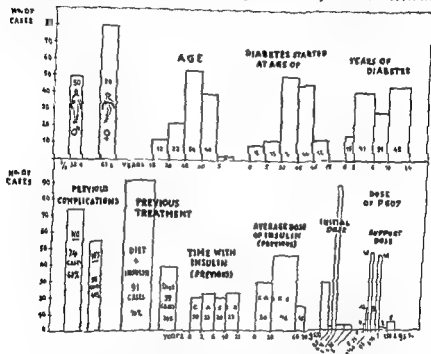


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ence of acetonemia, complete urinalysis, protidemia, lipemia urea in the blood, liver function tests, hemogram, renal adequacy test and, in a sizable number of cases, liver biopsy puncture<sup>13</sup> In the hospitalized patients, a study of the carbohydrate metabolism was performed with the standard curves (Allbright's technique), intravenous glucose, intravenous insulin glucose, and adrenalin insulin In six cases the action of chlorpropamide in intravenous administration was studied

### *Dosage Schedule for Chlorpropamide*

The drug was administered in most of the cases in 2 doses (1 after breakfast and 1 after lunch) In some patients the drug was given after dinner The highest total dosage administered thus far is 71 gm, the longest duration of therapy, 130 days All patients are continuing on their respective dosage schedules

### *Techniques and Standards Used*

Addis counts were made by the usual technique Blood tests were made as follows urea modified enzymatic technique of Wrenn glucose the

TABLE 2  
DEFINITIONS OF STANDARDS OF CONTROL AND EVALUATION FOR CHLORPROPAMIDE

Control	Blood sugar mg % (Somogyi Nelson)	Total urine sugar gm	Acetone
Excellent	60-110	none	none
Good	111-130	To 5 per cent of the food glucides	none
Fair	131-170	To 10-20 per cent of the food glucides	none
No control	Higher levels	Higher levels	—
Efficient	Produces excellent or good control		
Partially efficient	Produces poor control Reduces insulin use no less than 50 per cent Has stabilizing action on glucose homeostasis		
Inefficient	Apparently no action on diabetes		

Somogyi Nelson technique, alkaline phosphatase, the Bodansky method blood proteins, the techniques of Calvary, Cohn, Wolfson and Ichiva cholesterol, the method of Bloor, Sheftel, and Hill, MacLagan, the original technique with adaptation of electrophotometric reading, Hanger, the original technique, including maintaining in the dark for 48 hours Kunkel, the original technique, blood bilirubin the technique of Mallory and Evelyn, erythro sedimentation, the technique of Westergren, and the cytological test, the usual technique

Designations for standards of effectiveness of chlorpropamide in the control of diabetes in relation to blood sugar and glycosuria are shown in TABLE 2 Diabetic groupings according to response to chlorpropamide are shown in TABLE 3

TABLE 3  
CLASSIFICATION OF DIABETICS

Group	Definition
1	Controllable with proper diet and insulin of as much as 20 U Insulin with drawal does not produce ketosis
2	Controllable with proper diet and insulin dosage of as much as 59 U Insulin total withdrawal even if less than 20 U produces ketosis History of ketosis exists
3	Controllable with proper diet and more than 60 U of insulin With or without past history of coma

## RESULTS

### General

The effects of chlorpropamide on blood sugar levels in normal individuals are shown in FIGURE 2 FIGURE 3 shows the effect on blood sugar levels of diabetics and FIGURE 4 the effect on the control of diabetes as indicated by blood sugar levels and glycosuria

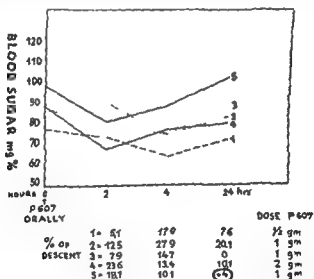


FIGURE 2 Glycemia response with chlorpropamide in five normal subjects

### Special

The complementary action of chlorpropamide on the control of cases of diabetes treated with insulin is illustrated in FIGURES 5 to 8 FIGURE 6 shows the control exercised by tolbutamide and chlorpropamide in diabetics undergoing surgery FIGURE 7 shows the action of chlorpropamide in two

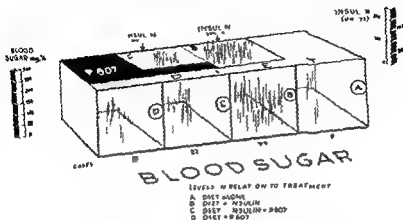


FIGURE 3 Blood sugar levels in relation to treatment

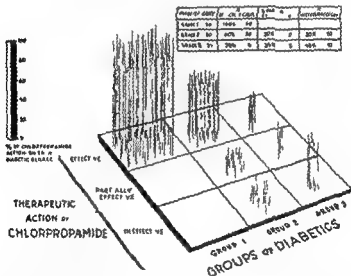


FIGURE 4 Results in 130 diabetic patients treated with adequate diet and chlorpropamide for 4 months

cases of pancreatic disease—one chronic pancreatitis with steatorrhea and diabetes the other with acute pancreatitis, operated for obstructive hepatitis (cholecystojejunostomy) three years before the beginning of diabetes. The action of chlorpropamide in one case of hyperthyroidism in diabetes is shown in FIGURE 8. Separate observations were made of the effect on blood sugar levels of methylthiouracil, methylmercaptomidazole, carbutamide, chlorpropamide, and chlorpropamide with methylthiouracil. In one case of



diabetes and vitiligo good control was possible with chlorpropamide (FIGURE 9) The effect on blood sugar level of chlorpropamide administered intravenously is shown in FIGURE 10

No beneficial effect was observed from chlorpropamide administered to two cases of moderate to intense acidosis Three cases of mild ketosis were correctable with the drug

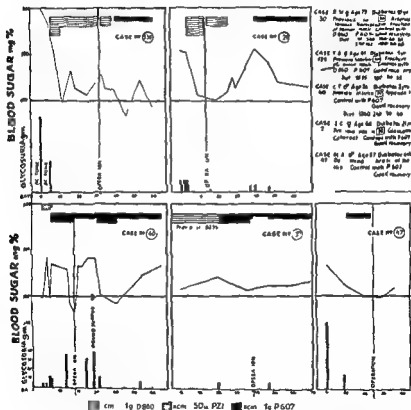


FIGURE 11: Surgical cases treated with tolbutamide and chlorpropamide

During treatment with chlorpropamide relatively high blood sugar levels were observed without glycosuria or with only slight glycosuria. This apparent elevation of the renal threshold is accompanied by a decrease in diuresis.

In two cases we have observed a relationship between dosage and degree

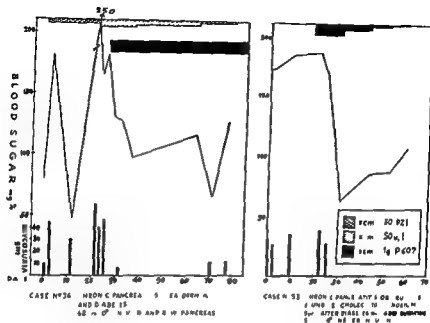
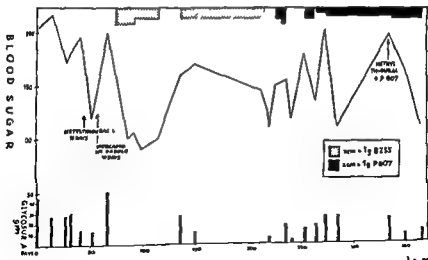


FIGURE 7 Act on of chlorpropamide on two cases of pancreatitis



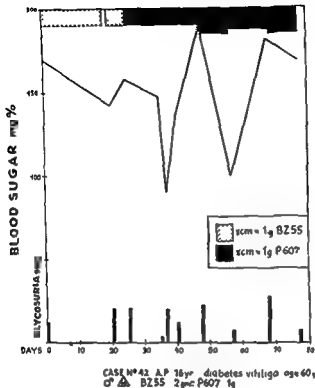


FIGURE 9 Action in relation to dosage

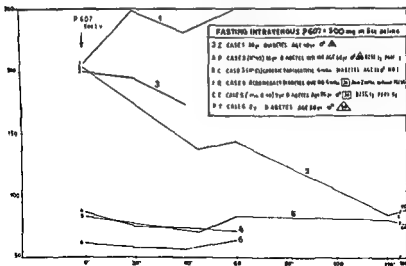


FIGURE 10 Effect on blood sugar level of intravenous administration of chlorpropamide



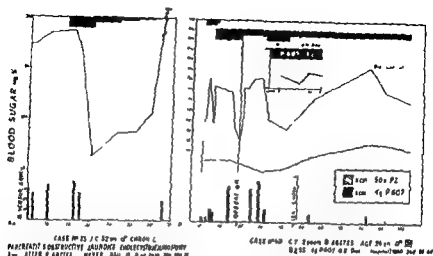


FIGURE 11. Action of chlorpropamide in relation to body weight and dosage

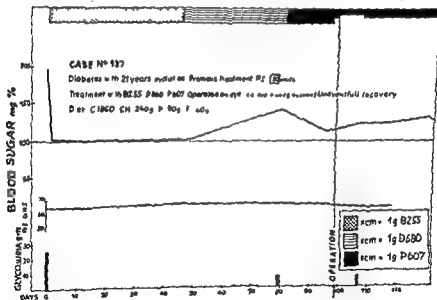


FIGURE 12. Action of drugs in patient whose body weight was kept constant

of the drug used and the degree of control in a patient in whom the body weight was maintained at a constant level (FIGURE 12)

#### *Side Effects in the Administration of Chlorpropamide*

There was 1 gastrointestinal case (constituting less than 1 per cent), and no renal or hepatic cases. Hematic effects were limited to 2 cases of leuko-

penia (1.6 per cent) and cutaneous effects to 15 cases (11.5 per cent), 13 of flushing (10 per cent) and 2 of pruritus with exanthema (1.6 per cent). The flushing observed appeared similar to that produced by the ingestion of nicotinic acid. In two cases where the flushing was intense, it was related

(0.8 per cent) of cyanosis that disappeared spontaneously and did not reappear when dosage was decreased from 0.5 gm to 0.25 gm of chlorpropamide. There were presented symptoms of reducing the dosage of chl symptoms of a mild hypoglycemia, but these cases were transitory and could not be classified definitely as actual hypoglycemic episodes. In one case (patient 26) epigastric and precordial discomfort was experienced 4 hours after ingestion of 0.5 gm of chlorpropamide, this coincided with a clearly low blood sugar.

Side effects were related to dosage. They appeared when the dosage was 1 gm daily or higher, and rarely with dosage of 0.5 gm daily (case 89, cyanosis). We have the impression that the margin between the therapeutic dosage and the dosage that produces side effects is small. In other words, the margin of safety is narrow.

## DISCUSSION AND CONCLUSIONS

### *Classification of Diabetes*

From our experience with sulfonylurea drugs and, particularly, with chlorpropamide in the treatment of diabetes, it is possible to subdivide diabetics into three groups. Our classification is based on symptoms that are obvious and reliable. After studying all symptoms that can be used for classification, it was decided that the most constant, in relation to the results obtained, are ketosis, diabetic coma, and previous insulin dosage required before establishing therapy with the sulfonylurea drugs. On the basis of the patients' responses with respect to these three factors, we have defined

TABLE 3

TABLE 4 shows the control achieved with other sulfonylurea drugs as observed in earlier studies carried out with the same methods used with chlorpropamide.

### *Administration of the Drug*

The minimum effective maintenance dosage of chlorpropamide was 0.125 gnt (in 4 cases) to 0.5 gm per day, the latter was the most commonly used dosage. One-half gram daily was effective in 38 cases. To be effective, it

was necessary to increase dosage to 1 gm daily in another 33 cases. When it was necessary to increase the maintenance dosage further, 1.5 gm was effective in 2 cases out of 3, and partially effective in 1 other. In 7 cases in which the drug was ineffective, the dose was increased to 2 gm, with some improvement. With chlorpropamide, the glucose-regulating effect is some

TABLE 4  
RESULTS WITH CARBUTAMIDE OR TOLBUTAMIDE IN 130 DIABETICS

Diabetic group	Carbutamide					
	Effective		Partially effective		Ineffective	
	Percent ages	No of cases	Percent ages	No of cases	Percent ages	No of cases
1	73.80	31	9.52	4	16.66	7
2	68.05	49	15.27	11	16.66	12
3	18.75	3		0	81.25	13
Tolbutamide						
1	61.42	43	17.14	12	21.42	15
2	48.07	25	34.61	18	17.30	9
3	37.50	3	25.00	2	37.50	3

times delayed for as long as 1 week or 10 days, during which time the patient should be observed carefully to determine if a change in dosage is necessary.

### Mode of Action

The investigation as conducted does not give any clue to the manner in which chlorpropamide acts. Its action appears to be similar to that of other sulfonylurea drugs, although effective dosage with chlorpropamide is lower than the dosages of the three other drugs.

Our investigations do not determine definitely all possible effects of long term therapy.

We have used chlorpropamide for five months, a rather short time, therefore we can reach only very preliminary conclusions.

*Tolerance of Chlorpropamide*

The tolerance of effective dosage has been good as the percentages of intolerance and of side effects indicate. Gastrointestinal intolerance increases with dosages greater than 1 gm daily.

*Usefulness of Chlorpropamide**Treatment with Chlorpropamide and the Importance of Dietary Measures*

Regardless of the group in which a diabetic is classified, a basic factor in the treatment is a balanced diet that normalizes body weight. It has been

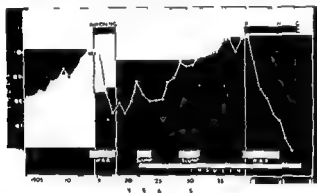


FIGURE 13 Comparative mortality indexes for diabetic patients in England and Wales

observed that with chlorpropamide as with other sulfonylurea drugs, stability is endangered when the patient's weight increases. Dramatic evidence of both has been furnished by the following example.

the mortality rate for diabetics dropped to a marked degree. No more graphic evidence of the importance of adhering strictly to dietary regimens exists.

## SUMMARY

Chlorpropamide was studied at the National Institute of Nutrition with the collaboration of diabetes centers of La Plata, Córdoba, and Mendoza. The medication was administered to a total of 200 diabetic patients, all more than 15 years of age. Thus far, however, detailed tabulations have been completed on only 130 of the cases. Patients were subdivided into 3 classifications:

Group 1 those controlled with insulin, dosage of as much as 20 U, but in whom withdrawal of insulin did not produce acidosis

Group 2 including (a) those controlled with less than 20 U of insulin, but in whom acidosis appeared on withdrawal, (b) those requiring between 21 and 59 U of insulin and (c) those presenting a history of acidosis

Group 3 those requiring more than 60 U of insulin or having a history of diabetic coma Using this classification of diabetic disease the results shown in TABLE 5 were observed with chlorpropamide in 130 patients of this series

TABLE 5  
RESULTS WITH CHLORPROPAMIDE IN 130 DIABETIC PATIENTS

Diabetic group	Total cases	Effective		Partially effective		Ineffective	
		Percent ages	No of cases	Percent ages	No of cases	Percent ages	No of cases
1	59	100	59	—	—	—	—
2	50	60	30	20	10	20	10
3	21	28	6	24	5	48	10

Hypoglycemic action has been demonstrated with chlorpropamide in normal individuals As for comparing its efficacy with carbutamide and tolbutamide equal hypoglycemic action was obtained with smaller doses of chlorpropamide Otherwise, the same factors appear to govern the effectiveness of these drugs Daily dosage ranged from 0.1 to 2.0 gm most frequently an initial dose of 1.0 gm (67 per cent of the cases), with 0.5 to 2.5 gm as additional doses included

Tests of hepatic and renal function revealed no disturbance, even in those patients receiving the drug for as long as 130 days The action of chlorpropamide has been studied in relation to a diet that contains less carbohydrate than a normal diet

#### ACKNOWLEDGMENTS

We express our appreciation of the collaboration of Boris Rothman, Vicente Osvaldo Perrone, Enrique Benito De Marco, Mario Calvagno,

#### References

1. LANDAUER, P. B. B. ROTHMAN & C. ALVARINAS. 1957. Acción de la BZ55 en los enfermos diabéticos. *Rev. asoc. arg. nutrición y dietol.* 67: 4.

- 2 LANDABURE P B B ROTHMIA & C ALVAREZ 1957 Acción de la D860 en los enfermos diabéticos. Rev asoc arg nutrición y dietol IV 34
- 3 CABARROU A, H A CAINO, O V PINTADO & N BIANCHI 1957 Preliminary experiences with D 860 in the therapeutics of diabetes mellitus. 17th Ann Meeting Am Diabetic Assoc. New York N Y
- 4 CAINO H A CABARROU, N BIANCHI & O V PINTADO 1957 Results obtained in La Plata Argentina in the treatment of 200 diabetic patients with BZ55. 17th Ann Meeting Am Diabetic Assoc. New York N Y
- 5 MARBLE A & R CAMERINI D'ALLOS 1957 Clinical experience with sulfonylurea compounds in diabetes. Ann N Y Acad Sci 71(1) 239  
MURPHY H H CAMERINI D'ALLOS & A MARBLE 1958 Results of long term use of tolbutamide (Orinase) in diabetes mellitus. J Am Med Assoc 167 818
- 6 DEBLE C 1958 Progresos en el estudio del uso de las sulfodrogas hipoglucemiantes por vía oral durante el último año. e Día Médico
- 7 DEBLE C & J SURAREMAN 1958 (April) Tratamiento con sulfodrogas en 156 diabéticos. Jornadas gastroenterol y nutrición Argentina
- 8 LANDABURE P B 1956 Aplicación s clínicas de las drogas hipoglucemiantes y antidiabéticas. Rev arg endocrinol y metabol 2 207
- 9 MONTAGNA C P R MONTEILLY J C GIORGIO & A SERANTES 1958 Experiencia clínica del Instituto Nacional de la Nutrición con sulfodrogas hipoglucemiantes en diabetes infantil y juvenil. Soc arg diabetes
- 10 WATMAN G & V ALVAREZ GARCIA 1958 Alteraciones morfológicas de la rata blanca producidas por la ingestión de carbutamida (BZ55). Asoc arg nutrición y dietol
- 11 WATMAN G & V ALVAREZ GARCIA 1958 Otras alteraciones físicas y fisiológicas producidas por la administración de carbutamida (BZ55) en la rata blanca. Soc arg diabetes
- 12 CABARROU A H CAINO F SCHAPACHIL I PIANZOLA & N BIANCHI 1958 Liver biopsies in diabetics treated with sulfonylureas. 3rd Congr Intern Diabetic Federation. Dusseldorf Germany
- 13 REGISTER GENERALS STATISTICAL REVIEW OF ENGLAND AND WALES 1947 Table Part I Medical H M S O London England

## PRELIMINARY CLINICAL AND EXPERIMENTAL STUDIES WITH CHLORPROPAMIDE IN DIABETES MELLITUS

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The purpose of this communication is to describe our experiences in the clinical management of sixty diabetic patients who have been treated with chlorpropamide for periods varying from a few weeks to six months. In addition the results of some comparative experiments on the action of tolbutamide and chlorpropamide *in vitro* are given. These suggest that the mode of action of the two drugs upon hepatic enzyme systems is similar but there seems to be a quantitative difference between them.

Clinical observations have been made on sixty adult diabetic patients twenty one males and thirty nine females. In view of earlier experiences

been made from patients attending the routine practice of the diabetic service of this hospital.

In contrast with earlier clinical trials conducted in the Diabetic and Metabolic Unit of the Alfred Hospital<sup>1,2</sup> many of the patients in this series were not admitted to hospital for continuous observation. It was felt that experience gained during the past three years was sufficient to enable us to assess the efficacy of chlorpropamide without the need for hospitalization. In consequence a number of patients were instructed to present the results of urine tests taken at regular times each day and spot blood sugar estimations

gations were undertaken for evidence of hepatic as well as renal abnormalities. Patients transferred from maintenance doses of insulin were instructed to report daily either in person or by telephone and to present the results of urinalyses performed four times each day.

Almost without exception the loading dosage prescribed was 1.0 gm per day and the maintenance dose 0.5 gm per day given in divided doses of 0.25 gm morning and evening. In any case that failed to respond the

number failing to do so. The relatively large number not responding within the first 4 weeks of treatment can be related to several such factors as inappropriate selection of patients and attempts to transfer cases from insulin therapy. TABLE 1 shows the distribution of the patients by age and sex

and TABLE 2 shows the over all results obtained in the entire series. It is convenient to present the results obtained for 4 categories of patients: (1) previously untreated, (2) previously treated by dietary restriction, (3) previously treated by diet and tolbutamide, and (4) previously treated by diet and insulin.

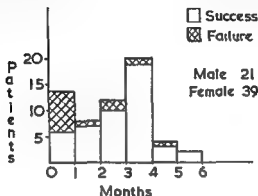


FIGURE 1

TABLE 1  
DISTRIBUTION OF PATIENTS BY AGE AND SEX

Age	Male	Female
20-30		1
30-40		1
40-50	2	1
50-60	4	5
60-70	9	15
70-80	4	13
80—	2	4
	21	39

(1) Fourteen patients previously untreated were placed upon an appropriate restriction of diet with chlorpropamide in an initial dosage of 1.0 gm. and a maintenance of 0.5 gm. per day. A number of these were assessed as likely to require insulin for control. Twelve patients responded satisfactorily, while 2 failed to do so.

(2) Twenty patients who had shown inadequate or intermittently satisfactory response to dietary restriction for periods from a few months to several years were treated along similar lines. Seventeen are considered to have shown a satisfactory response to chlorpropamide therapy, while 3 failed to do so.

(3) Four patients who had been treated for periods from 1 to 4 months



with dietary restriction and with tolbutamide in conventional dosages of 1.5 gm per day and all of whom had failed to show satisfactory response to this drug, were transferred to chlorpropamide in dosages of 0.25 gm twice daily. Three showed a satisfactory control while 1 failed to respond.

(4) Twenty-two patients whose condition had been regarded as controlled more or less satisfactorily by dietary restriction together with varying dosages of insulin were transferred to chlorpropamide therapy. Successful control was established in 15 while 7 failed to respond.

Chlorpropamide was used intermittently for periods of a few days to 1 to 2 weeks in treating still another group of 12 patients who, for various reasons, had become unstable while on dietary restriction alone. It was felt that a short trial of a sulfonyl drug might restore control and that the drug might

TABLE 2  
RESULTS OBTAINED IN ENTIRE SERIES

Previous treatment		Success	Failure
Nb	14	12	2
Diet alone	20	17	3
Diet plus tolbutamide	4	3	1
Diet plus insulin	22	15	7
	60	47	13

then be discontinued. In every instance the results obtained were considered entirely satisfactory as after a short course of dosages of 0.25 to 0.5 gm per day it was possible to discontinue its use, the patients being restored to their previous state of equilibrium.

TABLE 3 gives some details of the 13 patients who failed to respond. Two patients previously untreated, one a male of 54 and the other a female of 64, both with a history of diabetes of short duration who on general principles were considered likely to respond favorably, showed no evidence of control on dosages of 1.5 gm and 1.0 gm respectively although these dosages were continued for more than a fortnight. In neither case was there any evidence of an appreciable fall in the level of blood sugar.

In three instances, patients whose dietary control had been regarded as unsatisfactory for several months were tried with chlorpropamide therapy.

These patients were a female of 45 and a male of 58. They were treated for 6 to 11 weeks with chlorpropamide. In these patients, as in the others, the control was subsequently

satisfactorily controlled with insulin.

Seven of the 22 patients previously taking insulin failed to show satisfactory control when transferred to chlorpropamide. In 5 of these the duration of diabetes ranged from 6 to 10 years and their daily requirement of insulin varied between 20 and 50 U per day. In two instances, a female of 36 and

another female of 24, chlorpropamide was used because of the brittle nature of the diabetes, and in the hope that it might facilitate the action of insulin. In both instances it failed in less than 1 week, 1 patient developing a severe ketosis that necessitated admission to hospital.

Four patients who had been inadequately controlled by diet and tolbutamide were transferred to chlorpropamide. One patient failed to respond. She was 80 years old and, earlier in her diabetic history, had been controlled with 56 U of protamine zinc insulin each day. She had been transferred to 1.5 gm tolbutamide per day, but showed no response and, likewise, was not influenced by chlorpropamide.

In this trial there have been no unexpected episodes either in response or in failure to respond to this new sulfonyl compound. The side effects observed

TABLE 3  
DATA FOR THIRTEEN PATIENTS FAILING TO RESPOND TO TREATMENT

	Sex	Age	Wt	Dose	Duration	Side effects	Duration of diabetes
Previously untreated	M	54	159	1.5	2 weeks	Nausea	1 year
	F	64	155	1.0	2 weeks	—	4 months
Diet alone	F	73	124	0.75	3 months	—	4 months
	M	65	145	1.5	1 month	Nausea	2 months
	F	65	143	0.75	2 months	—	6 years
Diet and tolbutamide	F	80	200	0.50	5 months	—	3 years
Diet and insulin	F	63	177	1.0	2 weeks	—	12 yrs 40 NPH
	F	24	146	1.0	2 weeks	Ketosis	4 yrs 20 NPH
	F	52	146	1.0	2 weeks	—	6 yrs 24 NPH
	F	54	147	1.0	2 weeks	—	10 yrs 24 R 24 PZ
	F	74	86	1.0	1 week	Insulin allergy, nausea	20 yrs 40 NPH
	F	72	150	0.5	2 weeks	Diarrhea	1 yrs 28 R 20 PZ
	F	36	146	0.75	1 week	Ketosis	20 yrs 48 NPH

have been relatively insignificant. A small number of patients complained of nausea and this was more apparent in those cases in which the dosage was raised to more than 0.5 or 0.75 gm per day. In fact almost every case in which the dose was raised to more than 0.75 gm daily there was some complaint of nausea or dizziness. In 2 instances a photo-sensitive rash, no different from that observed in other forms of sulfonyl therapy, occurred and, in both this was of minor degree. On cessation of the drug the rash soon disappeared. In 2 instances albuminuria developed and the drug was discontinued despite the fact that there was no significant alteration in renal efficiency tests. It did not seem that any obvious renal damage had occurred. In all instances in which hepatic efficiency tests were performed there was neither clinical nor biochemical evidence that any hepatic disturbance was

occurring or had occurred as a result of chlorpropamide therapy. In no instance was any depression of blood formation observed. In three cases a curious effect was noted by patients after the consumption of preprandial alcoholic drinks, all complained of sensations of flushing of the face, which was obvious to onlookers and lasted for several hours.

In view of our previous experience that carbutamide and tolbutamide both apparently inhibit liver transaminase it was decided to investigate the effect of chlorpropamide and tolbutamide on the oxidation of  $C^{14}$  labeled amino acids by liver slices, using the drugs at a more physiological concentration than in previous enzyme experiments.<sup>2</sup>

The slices were incubated in Krebs phosphate buffer at a glucose concentration of 100 mg per 100 ml and a  $C^{14}$  DL alanine concentration of 40 mg

TABLE 4  
EFFECT OF TOLBUTAMIDE AND CHLORPROPAMIDE ON THE OXIDATION OF  $C^{14}$   
ALANINE BY LIVER SLICES

	No Series	$C^{14}O_2$ recovered as cpm/mg liver/hour	Difference	P
Experiment 1				
Control	6	108.5 $\pm$ 9.8 (S.L.M.)		
Chlorpropamide	6	154.7 $\pm$ 14.2	46.2	<0.01
Experiment 2				
Control	6	98.2 $\pm$ 3.4		
Tolbutamide	6	106.3 $\pm$ 3.4	8.1	<0.01

per 100 ml. Either chlorpropamide or tolbutamide was added to 1 of each pair of slices to give a final concentration of 30 mg per 100 ml.

The  $C^{14}O_2$  was collected on a KOH soaked paper spill in the center well of the Warburg vessel used, then eluted and precipitated as  $BaC^{14}O_3$ ; the radioactivity counted was corrected to infinite thinness. The results of this study are shown in TABLE 4. It is seen that both compounds accelerated the oxidation of the keto acid moiety of the amino acid in contradistinction to the result that might have been anticipated from the enzyme experiment.

Although these results appear at variance it is likely that the apparent inhibition of transaminase may well have been due to accelerated oxidation of the acceptor  $\alpha$ -ketoglutaric acid in the enzyme experiment, leading to a diminution in the amount of glutamic acid recovered. However, the suggestion that at least part of the action of these compounds is due to inhibition of neoglucogenesis is still valid, although the effect may be an acceleration of oxidation of Krebs cycle intermediates rather than an inhibition of their formation. It is interesting to note that at the same concentration chlorpropamide is roughly five times as effective in promoting the oxidation of alanine as is tolbutamide.

To practicing physicians, the discovery of the hypoglycemic action of

the sulfonylureas has provided a fresh interest in the management of diabetic

of diabetic patients. However it has been obvious for some time that control of hyperglycemia does not prevent patients from developing peculiar complications that affect the vascular and nervous systems. This has led to the speculation that diabetes mellitus is not always a problem of insulin deficiency alone and that substitution therapy with insulin does not prevent the development of complications at least in some patients. It is possible that physicians tend to forget that insulin has effects on the metabolism of proteins and of fats impossible to assess by any simple clinical means and that they place too much emphasis on easily performed tests for glucose in blood and urine.

Consequently when a chemical substance whose action is as yet unknown is found to be capable of reducing hyperglycemia in some patients and not in others it is not surprising that something resembling a therapeutic tumult has occurred. Eighty years ago Lancereaux<sup>6</sup> pointed out the clinical difference between *diabète maigre* and *diabète gras* a distinction emphasized by Himsworth<sup>8</sup> in 1949 and proved by Lawrence and Bornstein in 1950.<sup>7</sup> It seems that the sulfonylurea compounds are capable only of influencing those patients who are still able to produce some insulin of their own. Naturally fears have arisen that they may be disturbing bodily functions in a harmful fashion. Certainly it seems possible from the *in vitro* studies already

these compounds alter or even correct such a deviation from normal metabolism. This could offer an explanation for those cases whose hyperglycemia previously controlled with appreciable dosages of insulin is capable of control with small amounts of these new drugs given each day.

From this brief experience it would seem that chlorpropamide is capable of controlling the hyperglycemia of an appreciable number of diabetic patients who have developed the complaint in middle age.

No harmful side effects have been observed thus far in any of the patients treated with chlorpropamide.

There appears to be no qualitative difference between the patients who respond to carbutamide and those responsive to tolbutamide or chlorpropamide.

There appears to be a significant difference between the required dosages of chlorpropamide and tolbutamide in the patients who show response to the two drugs. This is also shown by *in vitro* studies on the effect of these drugs in the promotion of oxidation of alanine.

The suggestion is made that the action of these sulfonyl drugs is concerned with an acceleration of oxidation of Krebs cycle intermediates and that an inhibition of neoglucogenesis might explain their influence on some types of human diabetes.

occurring or had occurred as a result of chlorpropamide therapy. In no instance was any depression of blood formation observed. In three cases a curious effect was noted by patients after the consumption of preprandial alcoholic drinks: all complained of sensations of flushing of the face, which was obvious to onlookers and lasted for several hours.

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The slices were incubated in Krebs phosphate buffer at a glucose concentration of 100 mg per 100 ml and a  $C^{14}$  DL alanine concentration of 40 mg

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ALANINE BY LIVER SLICES

	No Series	$C^{14}O_2$ recovered as cpm/mg liver/hour	Difference	P
<i>Experiment 1</i>				
Control	6	108.5 $\pm$ 9.8 (S.F.M.)		
Chlorpropamide	6	151.7 $\pm$ 14.2	46.2	<0.01
<i>Experiment 2</i>				
Control	6	98.2 $\pm$ 7.4		
Tolbutamide	6	106.3 $\pm$ 3.4	8.1	<0.01

per 100 ml. Either chlorpropamide or tolbutamide was added to 1 of each pair of slices to give a final concentration of 30 mg per 100 ml.

The  $C^{14}O_2$  was collected on a KOH soaked paper spill in the center well of the Warburg vessel used, then eluted and precipitated as  $BaC^{14}O_3$ ; the radioactivity counted was corrected to infinite thinness. The results of this study are shown in TABLE 4. It is seen that both compounds accelerated the oxidation of the keto acid moiety of the amino acid in contradistinction

of the acceptor = ketoglutaric acid in the enzyme experiment, leading to a diminution in the amount of glutaric acid recovered. However the suggestion that at least part of the action of these compounds is due to inhibition of neoglucogenesis is still valid although the effect may be an acceleration of oxidation of Krebs cycle intermediates rather than an inhibition of their formation. It is interesting to note that at the same concentration chlorpropamide is roughly five times as effective in promoting the oxidation of alanine as is tolbutamide.

To practicing physicians the discovery of the hypoglycemic action of

the sulfonylureas has provided a fresh interest in the management of diabetic patients. For many years it has been thought desirable, if not essential, to control hyperglycemia and to reduce glyco-uria. The application of these principles has produced a remarkable improvement in the life and welfare of diabetic patients. However, it has been obvious for some time that

deficiency alone and that substitution therapy with insulin does not prevent the development of complications, at least in some patients. It is possible that physicians tend to forget that insulin has effects on the metabolism of proteins and of fats impossible to assess by any simple clinical means and that they place too much emphasis on easily performed tests for glucose in blood and urine.

Consequently when a chemical substance whose action is as yet unknown is found to be capable of reducing hyperglycemia in some patients and not in others it is not surprising that something resembling a therapeutic tumult has occurred. Eighty years ago Lancereaux<sup>6</sup> pointed out the clinical difference between *diabète maigre* and *diabète gras*, a distinction emphasized by Himsworth<sup>7</sup> in 1949 and proved by Lawrence and Bornstein in 1950.<sup>1</sup> It seems that the sulfonylurea compounds are capable only of influencing those patients who are still able to produce some insulin of their own. Naturally fears have arisen that they may be disturbing bodily functions in a harmful fashion. Certainly it seems possible from the *in vitro* studies already described that some deviation of normal metabolic processes occurs although thus far we have no evidence that this is either harmful or dangerous. If the diabetes of middle life is due to excessive neoglucogenesis it could be that these compounds alter or even correct such a deviation from normal metabolism. This could offer an explanation for those cases whose hyperglycemia previously controlled with appreciable dosages of insulin is capable of control with small amounts of these new drugs given each day.

From this brief experience it would seem that chlorpropamide is capable of controlling the hyperglycemia of an appreciable number of diabetic patients who have developed the complaint in middle age.

No harmful side effects have been observed thus far in any of the patients treated with chlorpropamide.

There appears to be no qualitative difference between the patients who respond to carbutamide and those responsive to tolbutamide or chlorpropamide.

There appears to be a significant difference between the required dosages of chlorpropamide and tolbutamide in the patients who show response to these drugs. This is also shown by *in vitro* studies on the effect of these drugs in the promotion of oxidation of alanine.

The suggestion is made that the action of these sulfonyl drugs is concerned with an acceleration of oxidation of Krebs cycle intermediates and that an inhibition of neoglucogenesis might explain their influence on some types of human diabetes.

*References*

- 1 DOWNIE E J BORNSTEIN M HUDSON & K TAYLOR 1956 *The role of sulphonyl urea derivatives in the management of diabetes mellitus—a preliminary report* Med J Australia 1 1072
- 2 DOWNIE E J BORNSTEIN & M HUDSON 1957 *Hypoglycaemic sulphonamides in the management of diabetes mellitus* Australian Ann Med 6 105
- 3 BORNSTEIN J 1957 *Inhibition of alanine transaminase by the hypoglycaemic sulphonylurea derivatives* Nature 179 534
- 4 LEVINE R 1957 *Concluding remarks* Ann N Y Acad Sci 71(1) 291
- 5 LANCERFAUX E 1880 *Chimie Interne Union Medicale* 29 161 205
- 6 HEMSORTH H 1949 *The syndrome of diabetes mellitus and its causes* Lancet 1 465
- 7 LAWRENCE R D & J BORNSTEIN 1951 *Two types of diabetes mellitus with and without available plasma insulin* Brit Med J 1 732

# THE EFFECT OF CHLORPROPAMIDE ON ALLOXAN DIABETES IN MICE\*

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Diabetes has been produced in mice by Kirschbaum and Bell<sup>1</sup> after subcutaneous injection of alloxan at the rate of 200 mg/kg body weight, and by Lazarow<sup>2</sup> after intravenous administration of 50 to 70 mg/kg. More recently we have reported<sup>3</sup> the differences in the diabetogenic effect of intravenously injected alloxan on different strains of mice. The present paper describes some preliminary observations about the effect of chlorpropamide† on the alloxan diabetic mice.

## Methods

Alloxan monohydrate (50 to 70 mg/kg body weight) was injected into the tail vein of adult male ZCeF<sub>1</sub> hybrid mice as previously described.<sup>3</sup> Blood samples taken by cutting the tip of the tail were analyzed for blood

weeks thereafter, eight of the surviving animals were normoglycemic, while the other ten were still severely diabetic.

Chlorpropamide was given by stomach tube once a day at a dosage of

with alloxan was used as control and treated with chlorpropamide simultaneously and in the same way.

## Results

The results are summarized in TABLE 1 which gives the mean blood sugar values (and standard error of the means) for the different groups of animals. The blood sugar values of the control animals before chlorpropamide was given are in the same range as those reported by Lazarow.<sup>2</sup> The blood sugar values of these animals did not change after the administration of chlorpropamide for 3 days (column 6 TABLE 1). A small decrease of 27 mg/100 ml was observed after 10 days of administration (column 7, TABLE 1), but this decrease proved to be not significant ( $t = 1.45$ ,  $p > 0.1$ ).

Similar response was observed in the group of animals with transient diabetes. The mean blood sugar value 10 days after chlorpropamide was 27 mg/100 ml lower but this decrease was statistically not significant ( $t = 1.1$ ,  $p > 0.1$ ).

\* The study reported in this paper was aided by grants from the Minnesota Division of the American Cancer Society, Inc., New York, N. Y., and the Public Health Service, Bethesda, Md.

† Supplied by Chas. Pfizer & Co., Inc., Brooklyn, N. Y.



In both groups there were animals that showed very marked decrease in blood sugar, 1 animal in the control group had a decrease of 93 mg /100 ml 10 days after administration of the chlorpropamide and 1 other, in the transient diabetes group, had a decrease of 135 mg /100 ml

In the diabetic group the administration of chlorpropamide produced a very marked decrease of the blood sugar 3 days after the beginning of treatment (column 6, TABLE 1). The mean decrease was 178 mg/100 ml, or

TABLE I  
EFFECT OF CHLORPROPAMIDE IN MICE<sup>a</sup>

Group	Number of animals	Mean blood sugar value and S.E. of mean†						
		5/29	6/5	6/26	6/30	7/7	7/14	7/21
Transient diabetes	8	314 ±45	401 +82	159 ±12	163 ±10	132 ±21	159 ±14	175 ±4
Permanent diabetes	10	383 ±36	561 ±52	334 ±11	156 ±19	165 ±4	183 ±7	234 ±24
Control	8	—	—	162 ±5	166 ±10	135 ±15	168 ±6	180 ±6

\* Alloxan was injected on May 14. Administration of chlorpropamide (100 mg/kg) began on June 27 and continued for 16 days through July 12.

† Upper figure of figure pair is mean blood sugar value in milligrams per 100 ml. lower figure is standard error of the mean.

All the animals had hypo-

### Comment

The results indicate a very marked hypoglycemic effect of chlorpropamide in the alloxan diabetic mice, which contrasts with the very slight effect observed in the control animal, and in those that were injected with alloxan but that were nondiabetic at the time of the administration of chlorpropamide. These observations are at variance with other observations regarding the effect of the sulfonylureas on the alloxan diabetic animal<sup>5,7</sup>. It would appear that chlorpropamide is more effective in decreasing the blood sugar of the diabetic mouse than in producing hypoglycemia in the normal animal.

## Summary

Twenty eight mice were injected intravenously with 50 to 70 mg of alloxan. All the animals developed marked hyperglycemia. Four weeks after injection of alloxan, 8 of the animals were normoglycemic. Chlorpropamide was given to these animals by stomach tube at a dosage of 100 mg/kg of

body weight as well as to 10 others that were still diabetic, and to 8 normal controls. Three days after the administration of chlorpropamide, the alloxan diabetic mice showed a very marked decrease of their blood sugar level. No significant effect was produced in the other two groups of animals.

### *References*

- 1 KIRSCHBAUM A & E T BELL 1948 *Experimental Diabetes Mellitus* Thomas Springfield Ill
- 2 LAZAROW A J 1947 *J Lab Clin Med* 32 1258
- 3 MARTINEZ C F GRANDE & J J BITTNER 1954 *Proc Soc Exptl Biol Med* 87 236
- 4 NELSON N 1944 *J Biol Chem* 153 375
- 5 MIRSKY I A G IERISUTTI & R JINAS 1956 *Proc Soc Exptl Biol Med* 91 475
- 6 LANG S & S SHERRY 1956 *Metabolism* 5 733
- 7 HANDEK A & J SCHOLZ 1956 *Deut med Wochschr* 81 899

## THE EFFECTS OF TOLBUTAMIDE AND CHLORPROPAMIDE ON PATIENTS EXHIBITING JAUNDICE AS A RESULT OF PREVIOUS CHLORPROPAMIDE THERAPY

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undesirable side effects that occur. Jaundice as a complication of carbutamide therapy has been reported<sup>1,2</sup>. Recently jaundice has also been reported<sup>3</sup> as a complication in tolbutamide therapy. Chlorpropamide is closely related to the above compounds differing from them in having a chloride group substituted on the benzene ring and a propyl instead of a butyl group at the end of the chain. With such a compound, one might anticipate the same type of allergic reactions as have occurred with carbutamide and tolbutamide. In studying twenty diabetic patients for the hypoglycemic effects of chlorpropamide three cases of jaundice with associated nausea and vomiting and with an erythematous maculopapular rash were encountered. The following case reports are illustrative.

*Case 1* D. J., a 58 year old Negro female, has had known diabetes mellitus since 1949 and was controlled on diet only until February 1958. Because of glycosuria, an elevated fasting blood sugar, and weight loss therapy was begun with 10 U of NPH insulin each morning before breakfast. Upon this regimen her diabetes became well controlled. On March 19, 1958 insulin was discontinued and chlorpropamide therapy was instituted with a dose of 0.5 gm twice daily. The day before a complete blood count was normal with no eosinophilia present. Urinalysis was negative except for a 1+ sugar. Liver function tests were normal. The thymol turbidity test was 3.3 U, the alkaline phosphatase test was 3.78 Bodansky units and the total serum bilirubin was 0.28 mg per cent with a direct bilirubin of 0.13 mg per cent.

Initially, the patient tolerated the medication satisfactorily and was managed with the same degree of control as with the previous insulin regimen. During the third week of chlorpropamide therapy she developed anorexia, nausea and vomiting, a dark urine, pruritus and a skin rash. She stopped her medication on April 12, 1958. She was seen in the diabetes clinic on April 18 with the above complaints and was admitted to the Medical Service at Grady Memorial Hospital. Careful questioning revealed no history of exposure to rats or hepatotoxic agents, or to anyone with hepatitis. Needle punctures were limited to her own insulin syringe and needle used by no one else and to those done in our laboratory with careful precautions.

Upon examination the patient a temperature of 103° orally, the pulse rate was 116, and a generalized maculopapular rash of

were no mucous membrane lesions, the sclerae were icteric, and there were grade 2 arteriosclerotic changes of the fundi. The lungs were clear. The heart was slightly enlarged to the left; a grade 2 apical systolic murmur was recorded. The abdominal examination revealed no evidence of hepatomegaly. No organs or masses were palpated. The remainder of the examination was noncontributory.

When the patient was admitted, the blood count showed a Hb of 12.4 gm, Hct 41 and WBC 3900 with 14 per cent bands, 57 per cent neutrophils, 6 per cent eosinophils, 17 per cent lymphocytes, and 6 per cent monocytes. Platelets were adequate. By April 30 the Hb had dropped to 10.5 gm and the Hct to 32.5. WBC was 9400 with 13 per cent eosinophils, 46 per cent neutrophils, 2 per cent bands, 6 per cent metamyelocytes, 31 per cent lymphocytes, and 2 per cent monocytes. By May 27 the blood picture had returned to normal revealing a Hb of 11.8 gm, Hct 35 and WBC 6700 with 4 per cent eosinophils, 56 per cent neutrophils, 4 per cent bands, and 36 per cent lymphocytes.

Urinalysis on admission of the patient revealed a trace of sugar, 4+ acetone, and 4+ bile. The  $\text{CO}_2$  combining power was 23.9 mmoles/l. The 4+ acetone was thought to have been determined on the basis of starvation. Liver function tests on April 18, 1958, revealed a total protein of 7.1 gm, albumin 3.9 gm, and globulin 3.2 gm, with an albumin globulin (A/G) ratio of 1.22. The thymol turbidity test was 2.25 U, and the cephalin flocculation test was 1+. The alkaline phosphatase test was markedly elevated at 26.9 Bodansky units. The serum bilirubin was elevated to 10.7 mg per cent, with a direct bilirubin of 7.65 mg per cent. The prothrombin time of the patient was 43.4 sec, with a control value of 11.4 sec at 100 per cent, 19.8 sec at 30 per cent, and 26 sec at 20 per cent.

After the intravenous administration of 50 mg of vitamin  $\text{K}_1$ , the prothrombin time of the patient on April 19 was 14.0 sec, with a control value of 12.4 sec at 100 per cent, 21 sec at 30 per cent, and 27.6 sec at 20 per cent. On April 21 the prothrombin time was 15.0 sec, with the same control values as on April 19. On April 28 it was 13.0 sec, with a control value of 13.4 sec at 100 per cent, 21.0 sec at 30 per cent, and 29.0 sec at 20 per cent. Serum electrophoresis on April 25 revealed a non-specific elevation of the serum globulins.

By April 24 the alkaline phosphatase had dropped to 16.1 Bodansky units. After the patient was discharged from the hospital, the alkaline phosphatase was 5.66 Bodansky units on May 20, 4.33 on May 27, 6.88 on June 3, and 3.84 on June 10, 1958. Since the last date the  $\alpha_2$  value have remained normal.

On April 21 the total serum bilirubin was 8.5 mg per cent, with a direct bilirubin of 6.1 mg per cent. On April 22 the total serum bilirubin was 10.9 mg per cent, with a direct bilirubin of 7.9 mg per cent. On April 28 total serum bilirubin was 6.3 mg per cent, with a direct bilirubin of 5.0 mg per cent. After the patient was discharged from the hospital on May 20, the total serum bilirubin was 2.45 mg per cent, with a direct bilirubin of 1.6 mg per cent. On May 27 the total serum bilirubin was 1.5 mg per cent.

per cent      On June 3 the total serum  
per cent  
1

range

While the patient was in the hospital and at the time of the biopsy, the serum proteins, albumin globulin ratios, cephalin flocculation tests, and thymol turbidity tests have been within normal limits.

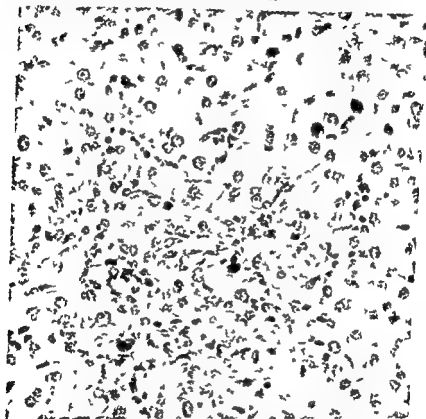


FIGURE 1 Hepatocellular damage as revealed by biopsy  $\times 160$

The biopsy was obtained on April 22, 1958. FIGURE 1 shows the micro-  
graph. There was a marked increase in mitotic activity.

The pattern of hepatocellular damage was present. There were also smaller canaliculi and hepatocellular damage. A chest X-ray was suggestive of slight cardiomegaly, but was otherwise negative.

For the first 4 days in the hospital a daily temperature elevation as high as  $102.2^{\circ}$  to  $103.8^{\circ}$  occurred. From the sixth through the eighth day the

temperature did not exceed 100° orally, and the patient remained afebrile for the remainder of her hospital stay. For the first 2 days she received parenteral fluids and insulin. By the third day she was placed on a 1200 Cal diet of 125 gm carbohydrate, 60 gm protein, and 40 gm fat, which was increased to 1800 Cal as 180 gm carbohydrate, 80 gm protein, and 84 gm fat when her appetite returned to normal. A fasting blood sugar was 95 mg per cent on April 22. With diet and 10 U of NPH insulin each morning before breakfast she remained aglycosuric and was discharged as improved on this regimen on May 7, 1958.

After her complete recovery, insulin was discontinued and tolbutamide therapy was begun on June 21, the patient receiving 3.0 gm the first day, 2.0 gm the second day, and 1.0 gm daily thereafter. She remained asymptomatic. Weekly blood counts, urinalyses, thymol turbidity tests, cephalin flocculation tests, A/G ratios, alkaline phosphatase tests, and total and direct serum bilirubin values remained normal. Tolbutamide was continued for 7 weeks until August 9, 1958, when chlorpropamide was substituted at a dosage of 250 mg daily. On this reduced dosage of chlorpropamide she has remained asymptomatic and aglycosuric, with normal blood glucose levels for the 5 weeks she has been on the drug. Weekly blood counts, urinalyses, thymol turbidity tests, cephalin flocculation tests, A/G ratios, alkaline phosphatase tests, and total and direct serum bilirubin values have been determined and have been found to remain within normal limits.

**Case 2** O.D., a 68 year old Negro female was discovered to have diabetes mellitus in December 1955, with the typical symptoms of polyuria, polydipsia, polyphagia, a 15-lb weight loss, lack of energy, and pruritus vulvae for 8 months. Her diabetes was treated with diet and 30 U of NPH insulin each morning before breakfast. On October 3, 1956, she was admitted to the Surgical Service of Grady Memorial Hospital for cholecystectomy. At the age of 26 years she had had typhoid fever followed by jaundice. In April 1956 the patient developed right upper quadrant pain and associated nausea and vomiting. Cholecystogram at this time revealed nonopacification of the gall bladder and cholelithiasis. Because of continued symptoms of indigestion and intermittent attacks of upper abdominal discomfort with radiation into the right scapular region, cholecystectomy was performed. Although her sclerae were thought to be slightly icteric on October 4, 1956, the total serum bilirubin was 0.3 mg per cent with a direct bilirubin of 0.2 mg per cent. At the same time the total protein was 6.4 gm, albumin 3.1 gm, and globulin 3.3 gm. The A/G ratio was 0.94, thymol turbidity was 4 U, and the cephalin flocculation test was 3+. These tests were repeated on October 5, 1956. The total serum protein was 7.6 gm, albumin, 3.1 gm, and globulin, 4.5 gm. The A/G ratio was 0.69, thymol turbidity was 4 U, and the cephalin flocculation test was 2+. The patient withstood cholecystectomy uneventfully and was discharged from the hospital October 18, 1956 on an 1800 Cal diet of 175 gm carbohydrate, 70 gm protein, and 91 gm fat with 35 U of NPH insulin each morning before breakfast. Her case was followed at the diabetes clinic and, on January 4, 1957, the NPH

and chlorpropamide therapy was instituted with a dose of 0.5 gm twice daily. The day before a complete blood count was normal, urinalysis was negative (except for a 4+ sugar), liver function tests were normal, the thymol turbidity test was 4.3 U, the alkaline phosphatase test was 3.7 Bodansky units and the total serum bilirubin was 11.35 mg per cent with a direct bilirubin of 0.15 mg per cent.

Initially the patient tolerated the medication satisfactorily and was managed with the same degree of control as with the previous insulin regimen. On the fourteenth day of chlorpropamide therapy the patient developed nausea and vomiting and a dark urine that persisted for 3 days (until April 18, 1958) when she was admitted to the hospital. Careful questioning revealed no history of exposure to rats or hepatotoxic agents or to anyone with hepatitis. Needle punctures were limited to her own insulin syringe and needle used by no one else and to those done in our laboratory with careful precautions.

At admission the lungs were clear and the heart sounds were normal. Abdominal examination revealed a cholecystectomy scar. The liver edge was felt two finger breadths below the right costal margin and was nontender. The remainder of the examination was noncontributory. The urinalysis on admission was negative except for the presence of bile. The blood count was within normal limits. Liver function tests on April 18, 1958 revealed a total protein of 7.3 gm, albumin 3.4 gm, globulin 3.9 gm and an A/G ratio of 0.87. The thymol turbidity test was 4.2 U and the cephalin flocculation test was 1+. The alkaline phosphatase test was elevated to 8.5 Bodansky units. The serum bilirubin was elevated to 6.35 mg per cent with a direct bilirubin of 4.25 mg per cent.

The prothrombin time of the patient was 39.2 sec with a control value of 11.4 sec at 100 per cent, 19.8 sec at 30 per cent and 26.0 sec at 20 per cent. Following the intravenous administration of 50 mg of vitamin K, the prothrombin time on April 19 was 15.0 sec with a control value of 12.4 sec at 100 per cent, 21.0 sec at 30 per cent and 27.6 sec at 20 per cent. On April 21 it was 14.2 sec with the same control values as on April 19. On April 28 it was 13.4 sec with a control value of 13.4 sec at 100 per cent, 21.0 sec at 30 per cent and 29.0 sec at 20 per cent. On April 22, 1958 the

it was 7.8 Bodansky units. Weekly determinations since then have been normal.

On April 22 1958 there was an increase in reversal of the A/G ratio, with a total protein of 7.2 gm albumin 3.1 gm globulin 4.1 gm and an A/G ratio of 0.76. On April 25 serum electrophoresis revealed a nonspecific increase in globulins. On April 28 the total protein was 7.7 gm albumin 4.5 gm globulin 3.2 gm and an A/G ratio of 1.4. Weekly A/G ratios have remained normal. A Bromsulphalein test on April 28 revealed 10 per cent retention of dye after 45 min. This was repeated on July 15 1958 with a retention of 4 per cent.

A liver biopsy was obtained on April 23 1958 after the patient had almost completely recovered. The biopsy revealed minimal hepatocellular degeneration minimal parenchymal cell jaundice and minimal pericholangitis with bile stasis.

A chest X-ray was negative. While in the hospital the patient remained afebrile. By the following day the nausea and vomiting had subsided and a sense of well being returned. The diabetic state was well controlled on an 1800 Cal diet of 175 gm carbohydrate 70 gm protein 91 gm fat and 30 U of NPH insulin each morning before breakfast. She was discharged without complaints on April 28 1958.

One month after complete recovery insulin was discontinued. tolbutamide therapy was begun on June 1 1958 in dosages of 3.0 gm the first day 2.0 gm the second day and 1.0 gm daily thereafter for 7 weeks. Weekly blood counts urinalyses thymol turbidity tests cephalin flocculation tests A/G ratios alkaline phosphatase tests and total and direct serum bilirubin values have remained normal. The cephalin flocculation test was 3+ on 2 occasions but negative to 2+ in the remaining tests. She remained asymptomatic and fairly well controlled on this regimen. On July 19 1958 tolbutamide was discontinued and chlorpropamide was begun with a daily dosage of 250 mg. For 8 weeks she has remained asymptomatic and is now essentially aglycosuric. On August 28 1958 the fasting blood sugar was 137.5 mg per cent and a blood sugar 2 hours after breakfast was 175 mg per cent. Weekly blood counts urinalyses cephalin flocculation tests and total and direct serum bilirubin values were normal.

**Case 3.** L. C. a 50 year old Negro female was seen at the eye clinic in December 1955 complaining of difficulty with vision in the left eye. She was found to have bilateral cataracts. When she was admitted on January 9 1956 for cataract extraction glycosuria was found and the fasting blood sugar was 282 mg per cent. Repeat fasting blood sugars were 313 mg per cent and 264 mg per cent. On a 1500-calorie diet of 160 gm carbohydrate 55 gm protein and 70 gm fat she developed ketosis before insulin treatment was started. After cataract extraction was accomplished on the left eye she was discharged.

At this time she was given 15 U of NPH insulin daily. At this time the blood sugar was 150 mg per cent. Since then the insulin dosage was reduced to 15 U of NPH with the continuation of a 1500 Cal diet. She maintained fairly good control of her diabetes on this regimen with occasional glycosuria. On January 16 1957 cataract



extraction was performed on the right eye. The patient was observed at occasional intervals and seemed to be under fairly good control with 15 U of NPH insulin each morning; the weight varied usually between 100 and 105 lb. On March 18, 1958 insulin was discontinued and the patient was

At this time the total serum  
bilirubin of 0.14 mg per cent,  
phosphatase was 4.87 Bodan-

sky units. On March 28 the patient was aglycosuric and was feeling well, her weight was 105 lb. On April 18, 1958 anorexia, nausea, vomiting and generalized pruritus appeared and dark urine was noted for 2 or 3 days. Chlorpropamide was discontinued and 15 U NPH insulin each morning was again started on April 21, 1958. On April 25, 1958 she stated that the gastrointestinal symptoms disappeared as soon as she stopped taking chlorpropamide and the urine became normal in color about two days later, there was still some pruritus and her weight was 98 lb. The sclerae were mildly icteric and there was an erythematous maculopapular rash on the trunk. The liver and spleen were not definitely palpable. Careful questioning revealed no history of exposure to rats or hepatotoxic agents or to anyone with hepatitis. Needle punctures were limited to her own insulin syringe and needle, used by no one else, and to those done in our laboratory with careful precautions. On April 28 the total serum bilirubin was 1.85 mg per cent, with a direct bilirubin of 0.9 mg per cent. The thymol turbidity test was 1.45 U, alkaline phosphatase test was 13.2 Bodansky units, cephalin flocculation test was 1+, and A/G ratio was 0.92. The prothrombin time was normal. All symptoms and signs cleared rapidly so that on April 30, 1958 she appeared well, except for slight scaling of the skin over the areas of the abdomen previously involved by the rash. There was occasional glycosuria on 15 U of NPH insulin daily and the weight was 98 lb. On May 30 the

Bodansky units A/G ratio was 1.41:1 cephalin flocculation was 1+ prothrombin time was normal and the BSP test showed 2 per cent retention

tests A/G ratios alkaline phosphatase tests and total and direct serum bilirubin values have remained normal

### Discussion

Although we can not rule out absolutely the coexistence of a viral hepatitis as the cause of the jaundice the presence of an erythematous maculopapular rash in all 3 patients and the presence of a 13 per cent eosinophilia in 1 patient suggest very strongly that the etiology of the jaundice was related to a hypersensitivity reaction to chlorpromazine. Many drugs have been suggested as producing jaundice. Probably the drug that now most commonly causes jaundice is chlorpromazine. J. H. Moyer *et al*<sup>4</sup> reported 1 case of obstructive jaundice without hepatocellular involvement in 500 patients treated. L. L. Hollister<sup>5</sup> reported 17 cases of jaundice due to chlorpromazine among a total of more than 900 patients treated with the drug. He estimates that the over all incidence of jaundice in patients treated for at least 1 week with chlorpromazine is probably between 1 and 2 per cent. The work of these investigators strongly suggests a hypersensitivity reaction as the cause of the hepatic complications. The clinical picture of fever gastrointestinal symptoms malaise and jaundice developing within 4 to 5 weeks of therapy together with the laboratory findings primarily of an obstructive type of jaundice is very similar to the findings in our cases.

Methyltestosterone as a cause of jaundice has also been described.<sup>6</sup> A. B. Gutman<sup>7</sup> has discussed the drug reactions characterized by cholestasis associated with intrahepatic biliary tract obstruction. He reported the following drugs as causative agents: arspenamine, dinitrophenol, cinchophen, aurothioglucose, methyltestosterone, norethandrolone (Nilevar), thiouracil, para-aminosalicylic acid, sulfadiazine, methimazole, 8 (para-aminobenzyl) caffeine. He considered these reactions to be the result of hypersensitivity to the drugs involved. F. A. Gibbs and F. W. Stamp<sup>8</sup> report serious liver damage occurring in about four persons in one thousand receiving phenurone

used as chemotherapeutic agents have been well known for some time.<sup>9, 10</sup> It is thought that a hypersensitivity reaction is responsible for these side effects although the exact mechanism of action on the liver is not definitely known.

The predominant picture in our cases was an obstructive jaundice with hyperbilirubinemia, an elevated alkaline phosphatase and only slight abnormalities in hepatocellular function. It was interesting that two of the

patients had a marked elevation of the prothrombin time. Another evidence that serious hepatocellular damage had not occurred was the prompt return to normal of the prothrombin time after vitamin K<sub>1</sub> therapy. Cholestasis was a prominent feature of the liver biopsy findings although hepatitis was also present. The similarity of our findings to the reports of hypersensitivity reactions with jaundice due to various drugs makes us feel that our cases definitely have this basis.

Because of a rather high incidence of cross sensitivity to the sulfonamides we attempted to determine whether these patients would show any side effects when placed on tolbutamide therapy. With an initial dose of 3.0 gm, a second dose of 2.0 gm, and a daily dose of 1.0 gm, there was no evidence of hypersensitivity. The patients remained asymptomatic, and weekly blood counts, urinalyses, and liver function tests remained normal for the 7 weeks they were kept on tolbutamide.

After tolbutamide therapy these patients were again placed on chlorpropamide but with a reduction of the dose from 1.0 gm to 250 mg daily. Two of the 3 have tolerated the drug without side effects for 5 and 8 weeks respectively. They have remained asymptomatic. Weekly blood counts, urinalyses, and liver function tests have remained normal. On the ninth day of resumption of chlorpropamide the third patient noticed nausea and vomited once. She immediately discontinued the drug on her own volition and resumed tolbutamide which she has continued to take without incident. At the time she stopped chlorpropamide no abnormalities by physical examination, blood count, urinalysis, or liver function tests could be found. We are not in a position to state whether these gastrointestinal complaints were related to the drug or were due to some unrelated cause. One might expect evidence of hypersensitivity when the patients were again administered the drug. In 1 of these patients it may have been the cause of the gastrointestinal complaints. Possibly the reduction in dosage may have decreased the likelihood of this reaction. With true hypersensitivity however one would have expected some evidence of hypersensitivity even with a smaller dose of the drug. L. E. Hollister<sup>3</sup> reported recurrence of jaundice in 6 of 11 patients treated with chlorpromazine, 9 of the 11 revealing some evidence of hypersensitivity. None of his patients showed cross sensitivity to chlorpromazine.

#### Summary

Three cases of jaundice as a hypersensitivity reaction to chlorpropamide have been presented.

The jaundice was primarily an obstructive type with hyperbilirubinemia and an elevated alkaline phosphatase.

There was no evidence of cross sensitivity to tolbutamide.

No recurrence of jaundice or evidence of impairment of hepatic function occurred when a smaller dose of chlorpropamide was given.

On the ninth day of resumption of chlorpropamide one case developed mild gastrointestinal symptoms. These symptoms cannot be definitely related to the drug.

# References

- 1 KIRKLEY W R 1957 Occurrence of sensitivity and side reactions following carbutamide *Diabetes* **6** 72-73
- 2 CAMERINO DI VALDES R H F ROOF & A MARBLE 1957 Clinical experience with carbutamide (N7 55) *Diabetes* **6** 74-77
- 3 MARBLE A 1959 Grand Rounds (televised) The Upjohn Co Kalamazoo Mich
- 4 MOYER J H, B KENT R W KNIGHT G MORRIS M DIXON S ROGERS & C SPEER 1954 Clinical studies of an antihemetic agent chlorpromazine *Am J Med Sci* **228** 174-189
- 5 HOLLISTER L J 1957 Allergy to chlorpromazine manifested by jaundice *Am J Med* **23** 870-879
- 6 WERNER S C J M HANZER & R A KRITZLER 1950 Jaundice during methyl testosterone therapy *Am J Med* **8** 325-331
- 7 GUTMAN A B 1957 Drug reactions characterized by cholestasis associated with intrahepatic biliary tract obstruction *Am J Med* **23** 841-845
- 8 GIBBS E A & F W STAMPE 1958 *Epilepsy Handbook* Thomas Springfield Ill
- 9 FRENCH A J 1946 Hypersensitivity in the pathogenesis of the histopathologic changes associated with sulfonamide therapy *Am J Pathol* **22** 679-687
- 10 MORE R H G C McMILLAN & G L DUFF 1946 The pathology of sulfonamide allergy in man *Am J Pathol* **22** 703-721

# STUDIES ON THE USE OF CHLORPROPAMIDE IN PATIENTS WITH DIABETES MELLITUS\*

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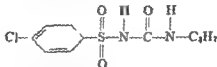
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The study of oral hypoglycemic agents has greatly stimulated research in diabetes mellitus. Many new compounds capable of producing hypoglycemia are being synthesized and studied in an effort to find one such compound that will prove to be as effective as insulin in controlling diabetes mellitus and yet still be suited to oral administration.

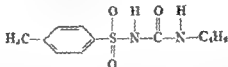
It is the purpose of this report to describe our clinical experience with chlorpropamide, a halogenated sulfonylurea compound, and to compare in detail the efficiency of this drug with that of insulin in managing patients with diabetes mellitus.

## *Chemistry and Pharmacology*

Chlorpropamide has the chemical name of 1 propyl 3 (p-chlorobenzene sulfonyl)urea and the chemical structure



Tolbutamide, in comparison has the chemical name of 1-butyl 3 tolyl-sulfonylurea and the chemical structure



The LD<sub>50</sub> in mice and rats is comparable with both chlorpropamide and tolbutamide according to one study, while another group reports that chlorpropamide shows greater toxicity. Both of these compounds showed similar side effects (depression, ataxia, tremors) in mice and rats, usually only at the higher dose levels.<sup>1,2</sup>

Acute, subacute, and chronic toxicity studies of this drug done on mongrel

\* The study reported here was supported in part by funds from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md., and Chas Pfizer & Co. Inc. Brooklyn, N. Y.

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dogs showed no significant anatomical changes either gross or histological, according to one group of investigators whereas a second group found liver degeneration and fatty infiltration as well as nephrotic changes in the kidney<sup>1,2</sup>

Blood level determinations were done on normal human volunteers. Three groups of these volunteers were used: equal numbers of volunteers were placed on dose levels of 0.25 gm per day, 0.50 gm per day and 1.0 gm per day of chlorpropamide. Each of these groups was observed to reach a mean plateau blood level of chlorpropamide on approximately the fifth day of drug ingestion. The volunteers receiving 0.25 gm per day reached 10 mg per cent, those receiving 0.5 gm per cent and those receiving 1.0 gm per cent.

At 1.0 gm per day of chlorpropamide became hypoglycemic thereby necessitating discontinuance of the compound. Blood levels were determined in these three after drug withdrawal and it was found that the removal of chlorpropamide from the blood stream was accomplished very slowly, suggesting that no marked active destruction of this compound occurs in the human body and that loss from the blood stream is accomplished by very slow renal excretion.

#### *Materials and Methods*

Fifty-two patients ranging in age from 25 to 88 years and with proved diabetes mellitus were maintained on chlorpropamide for periods ranging from 1 to 25 weeks. Forty-eight patients were observed at weekly or bimonthly intervals depending on the need for a visit.

Four patients were carefully studied in the hospital on calculated diets. Four others had been on no previous treatment prior to the administration of chlorpropamide. Forty patients had been previously managed with insulin therapy: five with tolbutamide and four by diet alone. Only two patients had been treated for diabetic acidosis prior to administration of chlorpropamide.

The selection of patients for the study was random although in some instances patients were begun on chlorpropamide because of frequent insulin reactions, poor control with insulin, inability to self-administer insulin or insufficient funds with which to purchase insulin (FIGURE 1).

All patients were placed on calculated diabetic diets but only those who were evaluated during hospital admission had careful control of diet. The clinic patients who constituted the lower economic stratum of the patients evaluated did not observe rigid control of their diets.

Clinic patients were evaluated for degree of control with chlorpropamide

urine examinations. All blood sugars were determined by the Folin Wu method.<sup>3</sup>

Twenty-three patients had serum protein bound iodine (PBI) determina-

tions made before or within seven days following the administration of the initial dose of chlorpropamide repeat PBI determinations were continued throughout the treatment period. The PBI determinations were made by a modification of the alkaline ash method of Barker.<sup>4</sup>

Twenty two patients had serum cholesterol determinations made before or within seven days following the administration of the initial dose of

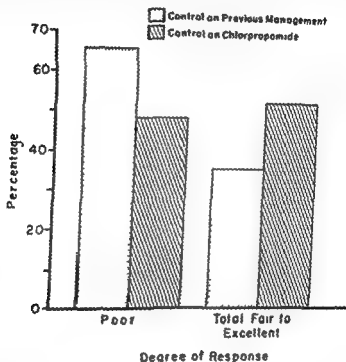


FIGURE 1 Comparison of the control of diabetes by chlorpropamide with previous management

chlorpropamide. These determinations were made using the method of Pearson.<sup>5</sup>

All patients had weekly to bimonthly urinalyses testing for red and white cells, albumin, specific gravity, sugar, and acetone. Three patients had repeated liver function tests using standard methods, that is, alkaline phosphatase, cephalin flocculation, and thymol turbidity. Four patients were evaluated with BSP tests. All patients were observed with total and differential white blood cell counts as well as packed cell volumes or hemoglobin determinations.

Initially, all patients were begun on 2 gm. of chlorpropamide daily. The dose level was then quickly decreased to 1.0 or 0.5 gm. per day. The maintenance dose in the majority of patients treated was 0.5 or 0.25 gm. per day and never exceeded 1.0 gm. per day for any patient. The various degrees of control of the diabetes mellitus in each patient both before and after chlor

propamide was administered and were designated as follows: excellent, 12 mg to 140 mg per cent; good, 120 to 140 mg per cent; fair, 140 to 160 mg per cent; and poor, more than 160 mg per cent.

FIGURE 1

*Efficacy in control.* The effectiveness of chlorpropamide therapy in controlling diabetes mellitus was evaluated on the basis of the patient's blood sugar level. The degree of blood sugar control was graded according to the average number of calories the amount of insulin required prior to

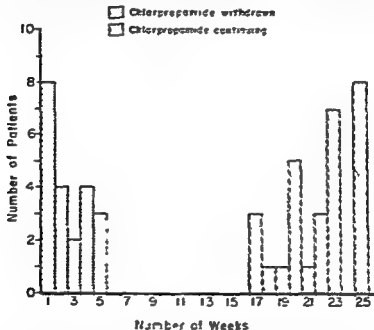


FIGURE 2. Duration of therapy with chlorpropamide.

treatment with chlorpropamide the duration of diabetes prior to chlorpropamide treatment, and the average weight during chlorpropamide treatment.

FIGURE 2 shows the duration of chlorpropamide treatment in all patients studied. Of 22 patients begun on chlorpropamide, 22 (100 per cent) were dropped by the end of 2 weeks of treatment, and 19 (86 per cent) were readmitted on treatment 1 to 25 weeks. It should be noted that no patient was readmitted within 1 week from chlorpropamide therapy after a week of treatment. Of the 22 patients readmitted to chlorpropamide treatment, 6 had poor control of blood sugar, 13 had fair control, 3 had good control, and 3 had fair to excellent control with a mean of 14.3 per cent.

FIGURE 3 shows separately the poor and the fair-to-excellent control



attained on the various means of prechlorpropamide treatment and on chlorpropamide treatment

TABLE 1 shows the degree of control of diabetes attained with chlorpropamide after 25 weeks of treatment. The over all control was fair to-excellent in 54 per cent and poor in 46 per cent of patients. While receiving various

TABLE 1  
DEGREE OF CONTROL OF DIABETES OBTAINED WITH CHLORPROPAMIDE AFTER 25 WEEKS OF TREATMENT

Age groups	Control				
	Excellent	Good	Fair	Poor	Total
21 to 30				1	1
31 to 40				2	2
41 to 50	2	1	1	3	7
51 to 60	6	2	1	4	13
61 to 70	7	1	4	8	20
71 to 80	1	2		5	8
81 to 90				1	1
Total	16	6	6	24	52
Per cent total	31.0	11.5	11.5	46	

TABLE 2  
CORRELATION OF BLOOD SUGAR CONTROL WITH THE AGE OF ONSET OF DIABETES MELLITUS

Age group (years)	Number of patients	Number of patients showing fair to excellent blood sugar response	Percentage of response
11 to 20	1	0	0
21 to 30	3	0	0
31 to 40	4	1	25
41 to 50	11	8	73
51 to 60	15	9	60
61 to 70	13	9	69
71 to 80	5	1	20

means of treatment prior to chlorpropamide administration, only 34 per cent of the 52 patients showed a fair to excellent response.

TABLE 2 shows a correlation of blood sugar control with the age of onset of diabetes mellitus. The 51 to 60 and 61 to 70 age groups had approximately the same percentage of fair to-excellent responses, with the 41 to 50 age group showing the best response.

TABLE 3 shows a correlation of blood sugar response with the duration of

diabetes prior to chlorpropamide treatment. There is essentially an equal number of patients in each group up to the 15 year duration group. Because of the small number in the groups after this, no accurate evaluation can be made. The 0 to 1 year and 1- to 5 year duration groups showed, in effect, an equal degree of control, as did the 6- to 10- and 11- to 15-year duration groups.

TABLE 3  
CORRELATION OF BLOOD SUGAR RESPONSE WITH THE DURATION OF DIABETES  
PRIOR TO CHLORPROPAMIDE TREATMENT

Duration of diabetes (years)	Number of patients	Number of patients showing fair to excellent blood sugar response	Percentage of patients responding
Less than 1	16	9	54
1 to 5	14	10	72
6 to 10	9	2	22
11 to 15	10	5	50
15 to 20	1	1	100
20 to 25	1	1	100
30 to 35	1	0	0

TABLE 4  
CORRELATION OF BLOOD SUGAR RESPONSE WITH AGES AT WHICH CHLORPROPAMIDE  
TREATMENT WAS BEGUN

Age group (years)	Number of patients	Number of patients showing fair to excellent blood sugar response	Percentage of response
21 to 30	1	0	0
31 to 40	2	0	0
41 to 50	7	4	57
51 to 60	13	9	70
61 to 70	20	12	60
71 to 80	8	3	38
81 to 90	1	0	0

TABLE 4 shows a correlation of blood sugar response with the ages at which chlorpropamide treatment was begun. Four of the age groups shown have approximately the same number of patients, and all have essentially the same blood sugar response, with a fair to excellent response ranging from 38 to 70 per cent.

TABLE 5 shows a comparison of blood sugar control while on chlorpropamide with that obtained on previous therapy. Again, there is a general tendency that suggests that the lower the previously required insulin dose, the better was the response to chlorpropamide. A 100 per cent response to

chlorpropamide was noted in the five patients previously controlled on tolbutamide

One hospitalized patient was carefully studied with both chlorpropamide and tolbutamide administration. During the testing of chlorpropamide the patient was observed with PBI determinations, cholesterol determinations, fasting, 11 A M, 2 P M, and 4 P M blood sugars, radioactive iodine<sup>131</sup> (RAI) uptakes, and liver function tests. This patient was an obese 65 year old Negro male with a history of diabetes mellitus of 20 years' duration. He had been well controlled on 35 U of NPH U-80 insulin per day when he was first discovered to have diabetes mellitus. He was then maintained on doses

TABLE 5  
COMPARISON OF BLOOD SUGAR CONTROL WHILE ON CHLORPROPAMIDE WITH THAT OBTAINED ON PREVIOUS THERAPY

Previous means of treatment	Number of patients	Number of patients showing fair to excellent blood sugar response	Percentage of patients responding
No insulin	9	4	45
1 to 20 U insulin	9	6	67
21 to 50 U insulin	13	7	54
51 to 100 U insulin	13	5	40
101 to 200 U insulin	2	1	50
Over 200 U insulin	1	0	0
Tolbutamide	5	5	100

of tolbutamide ranging from 1 to 4 gm per day, although excellent control required 4 gm daily.

FIGURE 3 shows the comparison of the control of this patient on tolbutamide and chlorpropamide after 10 weeks of study during each period. During both these 10 week periods dietary control was essentially the same. FIGURE 3 also shows that tolbutamide in doses of 4 gm per day was quite effective in maintaining the patient's blood sugar control in the range of excellent (below 120 mg per cent) while only a fair degree of control could be attained on 3.0 gm of tolbutamide per day. No untoward reactions to tolbutamide were recorded during this time. Also shown is the fact that

effective levels of the drug. Three weeks after the drug was discontinued the fasting blood sugar levels rose from 100 to 150 mg per cent and by the fifth week had risen to 163 mg per cent. It was concluded that the duration of action of chlorpropamide was approximately 3 weeks, and that normal blood sugar levels could again be attained within 1 week after starting 0.25 gm of chlorpropamide per day.

The first dose of 3.0 gm of chlorpropamide reduced this patient's blood sugar level from 110 mg per cent to 63 mg per cent in 5 hours. On the third day of therapy at this dose level a rather marked hypoglycemic reac

tion was produced during the early morning hours, with a blood sugar level of 57 mg per cent. The diabetes was well controlled on maintenance levels of 0.25 gm per day.

Mild side reactions were produced by 3.0 gm of chlorpropamide per day. They included minimal confusion and weakness, moderate somnolence, and minimal anorexia that persisted for 2 to 3 days following reduction of chlorpropamide to 0.25 gm per day, but rapidly disappeared thereafter. There

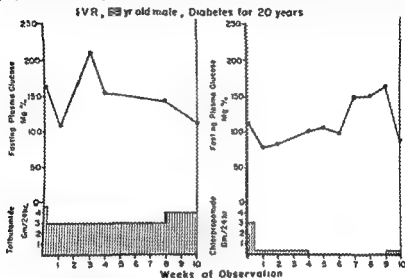


FIGURE 3 Comparison of the effect on fasting blood sugar levels of tolbutamide and chlorpropamide.

were no side reactions of any kind produced by the maintenance dose of 0.25 gm daily.

#### *The Effect of Chlorpropamide on Serum Cholesterol Levels*

Serum cholesterol levels were determined on twenty-two patients receiving therapeutic doses of chlorpropamide. All patients were placed on calculated diabetic diets. TABLE 6 shows the serum cholesterol levels obtained at various times from this group.

It is of interest that the serum cholesterol level of 9 (41 per cent) of the 22 patients decreased by 50 mg per cent or more while under treatment with chlorpropamide. The mean initial serum cholesterol level was 280 mg per cent, and the mean level after 25 weeks of treatment was 239 mg per cent—a mean decrease of 41 mg per cent, which includes the mean decreases in 13 patients who did not show a decrease of 50 mg per cent or more. There was a mean decrease of 79 mg per cent in the 9 patients who showed a 50 mg per cent or more decrease in serum cholesterol levels. The difference between these means was not statistically significant.

In almost all cases, the higher the serum cholesterol level prior to chlor

propamide administration the greater the fall in serum cholesterol level following chlorpropamide therapy. Evidence of this effect was further substantiated by the observation that in the 9 patients who showed a decrease in serum cholesterol of 50 mg per cent or more the mean serum cholesterol level prior to chlorpropamide therapy was 324 mg per cent in the 13 patients showing a decrease in serum cholesterol level of less than 50 mg per cent the mean initial level was 250 mg per cent. There was no correlation

TABLE 6

SERUM CHOLESTEROL DETERMINATIONS MADE AT VARIOUS WEEKS AFTER STARTING CHLORPROPAMIDE THERAPY

Patient	Pre treatment level 1 wk	Level weeks after therapy																			Mean following therapy
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	
1	222		231	205		253		231					248								234
2	304	272		226	225																241
3	352																				296
4		290	255																		225
5	324							303		192					218			242	221		285
6	238					200		181		243						309					189
7	306												206		194		179				177
8					224								147								185
9		294								256										244	250
10	232		245																		213
11	266						283		257	276	275			249							273
12	266		257		214			254						276							235
13	267														246						237
14	207		172	229		190															217
15	270			224		228		179													238
16	220					268		213							245						235
17	249								254												250
18	318			230			288	273		243											232
19	194			193				147	129								209				162
20	290					223	212		213										190		216
21	274	264		222																261	234
22	471			339				349	447												345
23	288				181	269															225
Mean	280																				239

between the degree of blood sugar control and the fall in serum cholesterol level nor was there a correlation between the fall in serum cholesterol the duration of diabetes or the amount of insulin previously required to control the diabetes

#### *Effect of Chlorpropamide on Thyroid Function*

Twenty three patients had repeated serum PBI levels while on chlorpropamide therapy. TABLE 7 shows the PBI determinations made at various weeks after starting chlorpropamide. The initial mean PBI level prior to chlorpropamide therapy was 5.8  $\mu$ g per cent, and the mean level following chlorpropamide therapy was 5.4  $\mu$ g per cent—a mean decrease of 0.4  $\mu$ g per cent. The difference in these means was not statistically

significant as a  
here

TABLE 7  
KIDNEY DETERMINATIONS MADE AT VARIOUS WELLS AFTER STARTING CHLORPROPAMIDF THERAPY

Several cases warrant discussion because of marked decreases in their serum PBI levels while on chlorpropamide therapy. One patient showed a gradual decrease in serum PBI levels from 5.6  $\mu\text{g}$  per cent to 3.6  $\mu\text{g}$  per cent, with levels falling to less than 3  $\mu\text{g}$  per cent on 2 occasions. A second patient showed a gradual decrease in serum PBI from an initial level of 6.0  $\mu\text{g}$  per cent to 4.3  $\mu\text{g}$  per cent after 19 weeks of therapy. Seven other patients showed a decrease in PBI levels of 1.0  $\mu\text{g}$  per cent or more while on chlorpropamide treatment. As far as could be determined, no symptoms referable to these decreases in PBI levels were produced.

In an effort to determine whether chlorpropamide depressed the 24 hour uptake of RAI, 5 patients had control 24 hour uptakes and uptakes following administration of large doses (3 to 7 gm) of chlorpropamide. RAI uptakes were also determined at hourly intervals and, when a plateau was reached, potassium thiocyanate (1.0 to 1.5 gm) was administered. The mean pre-chlorpropamide 24 hour RAI uptake in the 5 patients was 18.1 per cent, and the mean 24 hour uptake following chlorpropamide administration was 11.4 per cent—a mean decrease of 6.7 per cent. The difference between means was not statistically significant. There was no significant discharge of RAI following the administration of potassium thiocyanate in any of the patients. It appears, then, that chlorpropamide, at least in large doses, may be capable of suppressing the 24 hour RAI uptake but there is no evidence to suggest that chlorpropamide exerts any interfering action on the peroxidase enzyme system in a manner similar to propylthiouracil.

#### *Effect of Chlorpropamide on Blood and Urine*

All patients had complete peripheral blood evaluations and complete urinalyses at weekly intervals when first started on chlorpropamide therapy, the frequency of these determinations was decreased as therapy was prolonged.

At no time was there any evidence of anemia or granulocytopenia, although in a number of patients a slight increase in lymphocytes and monocytes was noted at approximately the same time the blood sugar fell toward normal. No pathological urine findings that could be attributed to chlorpropamide were noted in any of the patients.

#### *Effect of Chlorpropamide on Liver Function*

Three patients who had been maintained on relatively large doses of chlorpropamide for prolonged periods were evaluated with cephalin flocculation, thymol turbidity, and alkaline phosphatase tests. Four patients who had also been maintained for prolonged periods on large doses of chlorpropamide were evaluated with BSP tests. In none of these patients could any evidence of liver damage be found. Jaundice was not a finding in any of the fifty-two patients treated.

#### *Untoward Effects*

Although unpleasant to incapacitating side reactions occurred on all dose levels of chlorpropamide employed (0.25 gm every other day to 3.0 gm per

day), reactions were sufficiently severe in only 2 patients on 0.25 gm per day to warrant discontinuance of medication.

TABLE 8 shows the percentage of side reactions in patients receiving various amounts of medication. There was an over all incidence of side effects of some degree in 37 per cent of the patients, but only 15 per cent of the patients had sufficiently severe reactions to necessitate withdrawal of medication.

TABLE 9 reveals that weakness, hunger, dizziness, and somnolence were the most common complaints occurring on all dose levels used. The severity

TABLE 8  
PERCENTAGE OF SIDE REACTIONS IN PATIENTS RECEIVING VARIOUS AMOUNTS OF CHLORPROPAMIDE

Dose level* per day (gm.)	Number of patients	Number of patients having side reactions†	Percentage
3.00	3	2	67
2.00	13	12	92
1.50	2	1	50
1.00	13	7	54
0.50	39	24	62
0.25	33	15	47

\* Several patients are included at more than one dose level.

† The side reactions in patients receiving 0.50 gm. or less of chlorpropamide were usually mild and relieved by food.

TABLE 9  
ORDER OF FREQUENCY OF SIDE REACTIONS OBSERVED WITH CHLORPROPAMIDE THERAPY

1 Dizziness	7 Skin eruption
2 Weakness	8 Confusion
3 Hunger	9 Headache
4 Somnolence	10 Anorexia
5 Stasis	11 Vomiting
6 Nausea	12 Acidosis
13 Hypoglycemic coma	

of each of these 4 symptoms was related directly to the size of the dose used: the larger the dose, the more severe the complaint. These symptoms could not be correlated with hypoglycemia in all instances, but frequently—particularly in patients receiving 0.25 to 0.50 gm. of chlorpropamide per day—symptoms could be correlated with low blood sugar levels. Symptoms clinically resembling hypoglycemia could not be relieved by food ingestion when they were produced by 2.0 gm. per day or more of chlorpropamide, but could be relieved effectively by food ingestion when produced by doses of from 0.25 to 0.50 gm. per day. Dizziness, weakness, ataxia, somnolence and confusion usually occurred together, and at no time were they the sole complaints. In all cases these side reactions were most severe when they occurred on dose levels of from 1.0 to 3.0 gm. of drug per day, when produced



by doses of 0.5 to 0.25 gm daily they were rarely incapacitating. These effects were usually produced within hours of drug ingestion when they accompanied doses of 1.0 to 3.0 gm daily.

Severe somnolence was a complaint only in that group receiving 2.0 gm or more of chlorpropamide daily. These patients in the absence of preoccupation would immediately fall asleep, although they could be quite easily aroused and were not confused following arousal. Weakness was a prominent complaint in 2 patients receiving doses of chlorpropamide of 0.50 gm per day or less and could not be relieved by the ingestion of food.

Ataxia when it occurred, was usually present the day therapy was initiated but was maximal the second to third day of treatment. Many patients frequently fell as a result of it. Relief was always rapid and complete after marked reduction of the dose of chlorpropamide. Only 1 of 32 patients receiving 0.25 gm daily complained of ataxia; in this patient, the condition persisted for 1 week following withdrawal of the drug.

Nausea and vomiting occurred primarily in patients receiving 1.0 gm or more daily of chlorpropamide and was rapidly and effectively relieved by decreasing the dose used. At no time was vomiting sufficiently severe to . . . . . When nausea and vomiting . . . . .

Side reactions were commonly encountered in patients taking 0.25 to 0.50 gm of chlorpropamide daily. Of these reactions, headache and hunger were quite common and, even though they frequently occurred together they sometimes occurred independently. Characteristically they began late in the afternoon, just before the evening meal and would be completely relieved by the ingestion of food. Infrequently patients complained of being awakened by hunger during the early morning hours; food ingestion immediately prior to retiring prevented this effect. Somnolence when it occurred in patients receiving 0.25 to 0.50 gm of drug daily, frequently occurred after 6 to 8 weeks of therapy on chlorpropamide and appeared late in the afternoon, was associated with hunger and dizziness and was readily relieved by food.

Skin rash and pruritis were very common reactions, occurring in 12 of 71 patients (17 per cent) receiving 0.25 to 0.50 gm daily of chlorpropamide . . . . . while being treated. A definite . . . . . side therapy and the . . . . . was seen infrequently, as only 2 patients were maintained on such doses for more than 4 weeks. These 2 patients did not experience side reactions of any type, which suggests the marked individual variation of susceptibility to this drug.

Five of 39 patients receiving 0.5 gm of chlorpropamide daily complained of skin rash. The length of time patients were maintained on such a dose was considerably longer than the length of time patients were maintained on higher doses.

Seven of 32 patients taking 0.25 gm daily of chlorpropamide had skin rash

and pruritis at one time during the course of therapy. Both of these effects were usually noticed within 3 weeks after initiation of therapy and the incidence of these complaints increased as the duration of chlorpropamide therapy increased.

Skin rash characteristically was preceded or accompanied by pruritis although this was not a constant finding. A small percentage of the patients complained of skin rash alone. When pruritis preceded the skin rash it invariably did so by 1 to 3 weeks and did not persist following disappearance of the rash.

The rash was usually a maculopapular eruption erythematous discrete but never confluent. Characteristically the face (particularly the malar eminences), the neck, the upper third of the chest (both anteriorly and posteriorly) and the proximal third of the arms were the areas included. When pruritis developed first it was localized to these same areas. One patient developed a localized pretibial eruption. The skin rash was usually transient and of only 2 to 14 days' duration disappearing completely after this time. Two patients were exceptions to this one after being treated with 0.5 gm of chlorpropamide daily for 3 weeks developing an itching red rash. Chlorpropamide therapy was immediately withdrawn but the rash continued to increase in severity and 4 weeks later a severe exfoliative dermatitis developed with profuse vaginal and urethral discharge labial ulcers and mouth ulcers. The patient became extremely ill although following hospitalization for 2 weeks and extensive treatment she did well and was discharged without sequelae. A second patient was treated with 0.5 gm of chlorpropamide daily for 1 week. The drug was then withdrawn because of the development of nausea weakness ataxia and dizziness. One week after discontinuance of the drug the patient developed an itching, urticarial type of eruption that responded only minimally to extensive antihistaminic treatment. The rash progressed for 5 weeks and disappeared by the end of 7 weeks recovery again was complete.

Acidosis occurred in only two patients neither of whom had a blood sugar response to chlorpropamide. In both patients it developed two days after treatment was started. One patient was a juvenile diabetic with a past history of acidosis the other an adult diabetic without such previous history.

Hypoglycemic coma occurred in 1 patient a 60-year-old Negro male who had previously been poorly controlled on 130 U of NPH U 80 insulin daily. At the time of development of hypoglycemic coma he was receiving 1.0 gm of chlorpropamide per day. Symptoms developed during the early morning hours after he had failed to eat the evening meal the preceding night. On admission to the emergency room his blood sugar was 30 mg per cent. He responded immediately to intravenous glucose and has been well controlled since on 0.5 gm of chlorpropamide per day without further complaint.

Death occurred in one patient being treated with chlorpropamide but as this patient had her terminal treatment in an outlying hospital the details surrounding her death are unknown. An autopsy was not obtained but she had numerous severe complications such as congestive heart failure and chlorpropamide was thought not to be related to her death.

*Summary and Conclusions*

Studies are reported on 52 randomly selected adult patients with diabetes mellitus who were treated from 1 to 25 weeks with 0.25 to 3.0 gm daily of chlorpropamide. Thirty patients were maintained on this drug for from 17 to 25 weeks.

Fair to excellent blood sugar control was obtained in 28 (54 per cent) of the patients treated. The best response was obtained in those patients with a requirement of less than 21 U of insulin daily for control of the diabetes.

One patient was treated with both chlorpropamide and tolbutamide and  
 7 times as potent  
 No untoward

A mean decrease in serum cholesterol level of 41 mg per cent was observed in twenty two patients following treatment with chlorpropamide. This decrease was not statistically significant. A tendency for serum cholesterol to be lowered by chlorpropamide when initial serum cholesterol levels were high was suggested. The duration of diabetes, previous control of diabetes and the amount of insulin previously required for control played little or no part in determining the response of cholesterol to chlorpropamide.

Twenty three patients were studied to observe the effects of chlorpropamide on thyroid function. A mean decrease in serum PBI levels of 0.4  $\mu$ g per cent was observed following 25 weeks of treatment with chlorpropamide; this was not statistically significant. Five patients were observed to have a mean decrease of 6.7 per cent in 24 hour RAI uptakes following chlorpropamide treatment but again this was not statistically significant.

Untoward effects were common with chlorpropamide treatment and in general tended to be most severe and most frequent on dose levels of 1.0 gm or more daily. While these reactions were due to hypoglycemia in some instances and could be relieved by food ingestion in many instances they appeared to be the result of a direct effect of chlorpropamide. Side reactions were also commonly observed on maintenance dose levels of 0.25 to 0.50 gm daily but these were usually relieved by the ingestion of food and were thus transient and of little consequence.

Renal and hematological damage was not found with chlorpropamide therapy nor was liver function impairment observed.

*References*

1. C<sub>1</sub>
2. T<sub>1</sub>
3. Tolbutamide  
 J Biol Chem 181: 83
4. BROWN, H. A. W., RINGOLD & M. SAMOY. 1953. The determination of protein bound iodine by dry ashing. J Clin Endocrinol 13: 444.
5. PEARSON, S. S., STERN & T. H. MCGAVACK. 1953. A rapid accurate method for the determination of total cholesterol in serum. Anal Chem 25(4): 813.

# CLINICAL EXPERIENCE WITH CHLORPROPAMIDE IN THE MANAGEMENT OF DIABETES MELLITUS\*

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## Introduction

This report is based upon our observations of the compound 1-propyl-3-(*p*-chlorobenzenesulfonyl)urea (chlorpropamide) orally administered as an agent for the control of glycosuria and hyperglycemia as tested in 49 patients with diabetes mellitus for periods of 2 to 5 months. The drug has been found to be effective for this purpose in 76 per cent of this group, most of whom were patients whose diabetes began in their fifth decade or later. The data have been analyzed in terms of an arbitrary system for grading the degree of control achieved with the aid of the drug, and an appraisal on this basis of clinical factors influencing the effectiveness of the drug has been made. The concentration of chlorpropamide in blood serum attained at various dosage levels has been determined also, and the relation of these two factors to the effectiveness and toxicity of the drug has been evaluated.

## Clinical and Experimental Data

Of the 49 patients included in this study, 35 were females and 14 males. All but 6 of the patients previously had required insulin, and 14 of these had been treated with tolbutamide and had been adequately controlled by that compound. The remaining 6 patients were new diabetics, 2 having been controlled on tolbutamide prior to chlorpropamide administration; in all 6, the blood sugar levels had been constantly higher than 195 mg/100 ml before drug treatment, and good control was obtained by treatment in all 6.

Ten of the patients were studied initially for several weeks as inpatients in the Special Study Unit of Jackson Memorial Hospital. During the study they were kept on constant diet, and the excretion of glucose and ketones in the urine was determined quantitatively on partitioned 24-hour urine collections. Fasting blood sugar was also determined daily. After a suitable control period of observation, chlorpropamide therapy was begun, usually as 0.5 gm twice daily for two days, with subsequent maintenance on a single daily dose. Insulin, when it was being given during the control period, was withdrawn within 2 to 4 days of initiation of chlorpropamide therapy with initial daily decrements of about 50 per cent.

The same authors have published:

A. Gorman Hills and William A. Abelove, "Chlorpropamide in the Management of Diabetes Mellitus," *Ann. N.Y. Acad. Sci.*, 1957, 100, 1-12.

† Trautner (1957-1958) of the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

were controlled only insofar as they adhered to their diet prescription. They were seen at weekly intervals until their drug therapy and the response thereto were stabilized, and subsequently at intervals of two to three weeks.

If good control was achieved with 500 mg of chlorpropamide daily, the maintenance dose was lowered. If satisfactory control was not achieved with 500 mg daily, the dose was increased.

Determinations of chlorpropamide concentration in blood sera were made in both outpatients and inpatients with the aid of the method of Toolan and Wagner.<sup>1</sup>

TABLE 1 summarizes the status of the 49 patients with respect to previous treatment.

TABLE 1  
NUMBER OF PATIENTS ACCORDING TO PREVIOUS THERAPY

Insulin	29
Insulin then tolbutamide	14
Tolbutamide	2
New diabetics	4
Total	49

#### *Criteria of Control*

Criteria for satisfactory control were arbitrarily established as follows: good fasting true blood sugar consistently below 120 mg/100 ml, with no glycosuria; fair fasting blood sugar between 120 and 170 mg/100 ml, with glycosuria less than 0.5 per cent.

#### *Analysis of the Data*

*Age at onset of diabetes.* The effectiveness of chlorpropamide in controlling hyperglycemia is most striking in those patients in whom diabetes mellitus first made its appearance after the age of 40. Of 43 patients in this category who were given the drug, 36 or 84 per cent were adequately controlled without insulin. The excellent response often observed in these patients is exemplified in FIGURES 1 and 2.

In 5 patients whose diabetes began between the ages of 20 and 39, however, therapy was successful in only 1. The total lack of response encountered in patients of this group is illustrated in FIGURE 3. Chlorpropamide was also ineffective in the 1 child among our patients who received the drug, a 14-year-old boy with diabetes of 5 years' duration who required 52 U of lente insulin daily. TABLE 2 gives the age-at-onset data for the 49 patients.

variation in the sample for the number of patients is small and, as the age is confined to 4 of these patients who had had diabetes more than 20 years, the drug is shown effective for 75 per cent.

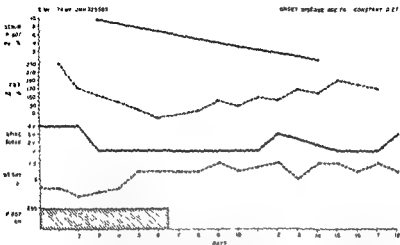


FIGURE 1 Response of a previously uncontrolled diabetic to chlorpropamide. After a control period on a constant diet, chlorpropamide therapy was begun without other

fasting blood sugar and P 607 to chlorpropamide

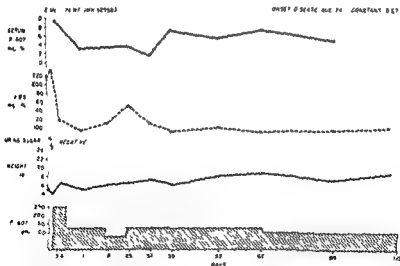
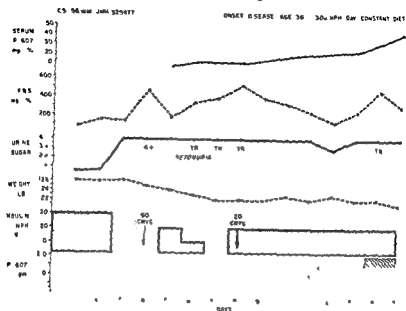


FIGURE 2 Effects of resumption of chlorpropamide therapy in the patient of FIGURE 1. Control for a 1½ month period has been excellent at a low dose level in this patient with newly diagnosed diabetes mellitus.



prophylactic (10-20 units daily) suppression of 20-40 units = 20-40 units

TABLE 2  
RELATION OF AGE AT ONSET OF DIABETES AND RESPONSE TO THERAPY

	Age at onset, years				Total patients	Percentage
	0-19	20-39	40-59	60-80		
No. of patients	1	5	22	21	49	—
Control						
Good	0	1	10	9	20	41
Fair	0	0	8	9	17	35
Unsatisfactory	1	4	4	3	12	24
Percentage satisfactory	0	20	82	86	—	—

*Previous insulin requirement.* There was no doubt that the effectiveness of chlorpropamide was related to the previous insulin requirement (TABLE 4). Good control was obtained in all patients who had not previously required

controlled. Our experience with those requiring more than 40 U of insulin daily was limited to two patients. One was the juvenile mentioned above,

the other a 68-year-old white female with an onset age of fifty, requiring 80 U of NPH insulin daily. On a daily dose of 500 mg chlorpropamide per day, her insulin requirement was reducible to 50 U of NPH daily, but no lower.

*Comparison of tolbutamide and chlorpropamide* In 15 patients previously maintained on tolbutamide in doses of 0.5 to 2.5 gm, chlorpropamide was substituted (TABLE 5). Thirteen of these had been on insulin prior to the tolbutamide. 2 were new diabetics. The effective dose of chlorpropamide

TABLE 5  
RELATION OF DURATION OF DIABETES AND RESPONSE TO THERAPY

	Duration of diabetes, years					Total patients	Percentage
	1	1-5	6-9	10-15	15		
No. of patients	7	18	6	12	6	49	—
Control							
Good	4	8	2	2	1	20	41
Fair	0	6	4	7	0	17	35
Unsatisfactory	2	4	0	3	5	12	24
Percentage satisfactory	71	78	100	75	50	—	76

TABLE 6  
RELATION OF INSULIN REQUIREMENT AND RESPONSE TO CHLORPROPAMIDE THERAPY

	Previous insulin dose, units per day				Total patients	Percentage
	0	Less than 20	20-40	Greater than 40		
No. of patients	6	13	28	2	49	—
Control						
Good	6	7	7	0	20	41
Fair	0	4	15	0	17	35
Unsatisfactory	0	2	8	2	12	24
Percentage satisfactory	100	85	71	0	—	76

averaged approximately one fifth that of tolbutamide, a typical experiment is shown in FIGURE 4. One patient (FIGURE 5) showing progressive resistance to tolbutamide was shifted to chlorpropamide, hyperglycemia and ketosis continued, and insulin was required for control.

*Sex and race* There was no correlation between the sex or race and response to therapy (TABLE 6).

*Dosage of chlorpropamide* The majority of our patients were maintained satisfactorily on doses of 500 mg per day or less, generally given as a single



TABLE 5  
COMPARISON OF TOLBUTAMIDE AND CHLORPROPAMIDE DOSAGE

	Previous maintenance dose (tolbutamide), grams per day					Total patients
	0.5	1.0	1.5	2.0	2.5	
No. of patients converted	1	3	6	4	1	15
Present maintenance dose (chlorpropamide) grams per day						
0.100	1	—	2	—	—	
0.200	—	2	3	—	—	
0.250	—	—	1	1	—	
0.400	—	1	—	1	—	
0.500	—	—	—	—	1	
0.625	—	—	—	2	—	

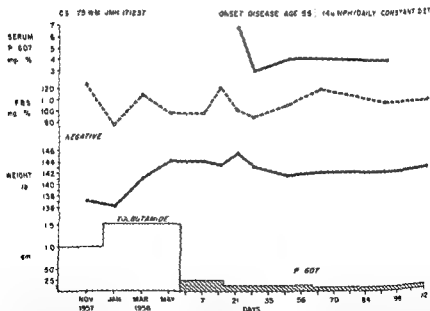


FIGURE 4 Replacement of tolbutamide by chlorpropamide. The observations illustrated were made while the patient was on a constant diet in the special study unit. Chlorpropamide, in a dose of 125 to 250 mg daily, maintained the satisfactory control previously achieved in this patient by 1 to 1.5 gm tolbutamide daily.

dose each morning. It was observed that patients taking small doses (that is 100 mg per day) could be controlled just as readily when the dose was given as a 200 mg tablet every other day (TABLE 7). In view of the relatively slow elimination of the drug it is probable that the same thing may be said of higher doses, but we have no observations to support this assertion.

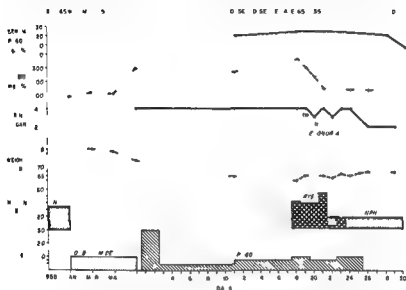


FIGURE 5. Failure of chlorpropamide in tolbutamide resistance. Neither tolbutamide (1 gm daily) nor chlorpropamide (750 mg daily) produced successful control of diabetes in this patient.

TABLE 6  
RELATION OF SEX AND RACE TO SUCCESS OF THERAPY

	Male	Female	White	Negro
No. of patients	11	35	37	12
No. successfully controlled	11	26	29	8
Percentage successfully controlled	79	74	78	67

Diabetics not responding to 500 mg per day usually failed to respond to doses of as much as 2 gm per day. At these higher dose levels toxic symptoms were not uncommon.

*Concentration of chlorpropamide in blood serum.* When chlorpropamide is administered in a constant daily dose of 1 gm or less, the serum concentration of the drug rises steadily for about five days, attaining a reasonably stable plateau by the end of that time. The decline in serum concentration when

the drug is stopped is still slower, and this is reflected in a rather delayed escape from diabetic control in patients who cease taking the drug (FIGURE 1)

FIGURE 6a represents the range of serum chlorpropamide concentrations found in a number of patients stably maintained at various dose levels of the drug. To these data has been fitted freehand the straight line curve shown in FIGURE 6b. The assumption, which may not be entirely accurate, has been made that the relation between drug dose and serum concentration is linear. At any rate FIGURE 6a gives a rough indication of the drug concentration to be expected at any dose level.

TABLE 7  
RELATION OF MINIMAL DOSE TO ADEQUATE CONTROL\*

	Chlorpropamide dose (grams per day)			Total
	0 1-0 2	0 25-0 50	0 60	
No. of patients	20	13	16	49
Control				
Good	14	6	—	20
Fair	6	8	5	17
Unsatisfactory	—	1	11	12

\* Satisfactory control was achieved in 33 of the 49 patients (67 per cent) by a daily dose

as 2 gm daily

TABLE 8 shows that satisfactory control, if it can be attained at all, will generally be achieved at a plasma concentration of chlorpropamide of

Thirty six of the patients represented in TABLE 8 have had repeated determinations of serum chlorpropamide concentration at a stable dose level of the drug, in 3 of these, a second series of observations was made at higher dose levels and with higher serum concentrations.

Of the 36 patients, 22 (61 per cent) were satisfactorily controlled at maximum serum concentrations of 15 mg or less per 100 ml. Three patients not satisfactorily controlled at the lower serum concentrations (15 mg/100 ml or less) were subsequently given larger doses of the drug. One remained uncontrolled with serum concentrations as high as 36 mg/100 ml, fair control was achieved in the other 2 at maximum levels of 20 and 25 mg/100 ml, respectively.

*Toxicity.* Toxic manifestations occurred in 15 of our 49 cases (30 per cent)

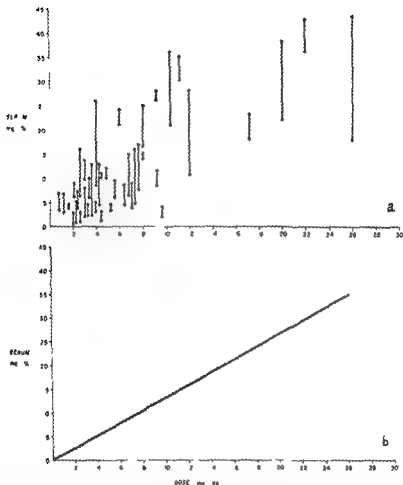


FIGURE 6. Relation of serum chlorpropamide levels to oral dosage. (a) Each bar shows the range of serum chlorpropamide concentrations attained 4 days or more after any given constant daily dose of the drug had been instituted. The data are based on 145 determinations in 39 patients; each range shown representing, on the average, 3.7 determinations carried out for 3 weeks or months at a stated dosage level. (b) A free hand curve drawn through the data of FIGURE 6a.

Most of the effects were transient and consisted of headache, dizziness, drowsiness, itching, and possible hypotensive symptoms (TABLE 4). There were, however, only 2 instances (4 per cent) where the symptoms persisted and necessitated withdrawal of the drug. In 1 patient (G. E.) who had been taking 100 mg per day with marginal fair control, the dose was increased to 50 mg per day, with ensuing nausea, vomiting, hiccoughs, and an erythem

15 mg/100 ml are reached. The maximum recommended dose is generally 8 mg/kg per day (500 mg per day for the average size patient). Further increases seldom give more effective control, and they will often raise the serum chlorpropamide level to a range higher than 15 mg, where a considerable increase of toxic reactions is to be expected. It is evident, however, that the dose of the drug per kilogram of body weight can influence effectiveness and toxicity only insofar as it affects the concentration of the drug in body fluids. Since the data show that there is considerable variation between individuals in the serum drug concentration attained at any given dose, it would appear that the determination of serum chlorpropamide concentration will often be helpful in establishing the optimal dose more precisely and, especially, in justifying increases to more than 8 mg/kg per day in patients who, on this amount of drug, may remain uncontrolled and maintain low serum drug concentrations.

The incidence of toxic reactions of some kind in our series has been high, but our figure of 30 per cent does not, of course, represent the incidence of toxicity to be expected in patients who will be treated after the principles of proper dosage have become well established. In the course of preliminary, experimental clinical studies of a new drug, neither the minimum effective dose nor the incidence of toxicity at various dose levels has yet become well defined, and the majority of the toxic manifestations reported here have occurred at a higher dosage than would now ordinarily appear to be indicated.

### Summary

Forty nine patients with diabetes mellitus, ranging from fourteen to eighty years of age have been treated with chlorpropamide for as long as five months.

Satisfactory control of the diabetes was achieved in 76 per cent of these cases, the majority of patients being those whose disease began after 40 and who required less than 40 U of insulin daily. The incidence of successful control was not significantly influenced by the sex or race of the patients nor by the duration of the diabetic state.

Chlorpropamide is effective in controlling diabetes, in general, under the same circumstances as tolbutamide, however, chlorpropamide is about five times as potent on a weight basis. This enhanced potency appears to

be due to the fact that the average concentration of chlorpropamide in the blood is about five times that of tolbutamide. A daily dose of 8 mg/kg for the average size patient is sufficient for control of the diabetes, but there is considerable variation, especially between individuals. For this reason, serum chlorpropamide determination should prove a helpful guide to therapy.

Toxic manifestations of some kind, principally mild and all reversible, occurred in 30 per cent of our cases. The incidence of toxic reactions in this

series is higher than and not representative of, that to be expected with therapeutic use of the drug which in our opinion should ordinarily consist of daily doses not in excess of 8 mg/kg of body weight

# Acknowledgment

We are indebted to Domenic G Iezzoni of Chas Pfizer & Co Inc Brooklyn, N Y for supplies of chlorpropamide

# References

- 1 TOOLAN T J & R L WAGNER JR 1959 The physical properties of chlorpropamide and its determination in human serum *Ann N Y Acad Sci* 74(3) 449-458
- 2 LEVINE R & G G DUNCAN Special Eds 1956 Symposium on clinical and experimental effects of sulfonylureas in diabetes mellitus *Metabolism* 5 721-972
- 3 ANNALS OF THE NEW YORK ACADEMY OF SCIENCES 1957 The Effects of the Sulfonylureas and Related Compounds in Experimental and Clinical Diabetes 71(1) 1-292

# CLINICAL AND EXPERIMENTAL STUDIES WITH CHLORPROPAMIDE IN DIABETES MELLITUS, IN NORMAL INDIVIDUALS, AND IN NONDIABETICS WITH HEPATIC DISEASE

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The hypoglycemic action of the sulfonylureas presupposes reserves of endogenous insulin. These substances probably also act through an extra pancreatic mechanism influencing intrahepatic metabolism, a process that has been confirmed clinically and experimentally by a selective accumulation of liver glycogen. Because the sulfonylureas produce no increase in sugar utilization by skeletal muscle tissue, their action differs significantly from the action that is typical of exogenous insulin. The liver, therefore, is one of the sites affected by sulfonylureas.

It has been clinically and experimentally shown that the sulfonylureas influence intrahepatic metabolism. The mode of action of chlorpropamide, as proved in our studies, does not differ significantly from that of carbutamide or tolbutamide. In test animals the antidiabetic drugs, including chlorpropamide, produce a similar increased accumulation of glycogen in the liver. In rats receiving 50 mg or 100 mg/kg of chlorpropamide (35 mg per day) exhibits

disease (hepatitis and cirrhosis), we observed a similar effect and, also an objective improvement in hepatic function, the latter was confirmed by laboratory and liver function tests, especially Bromsulphalein retention. Adverse effects on hepatic function were not demonstrated in these patients nor in 35 diabetics treated with chlorpropamide orally administered for several months. In rats receiving 50 mg or 100 mg/kg of chlorpropamide for 6 weeks, we observed an increase in liver glycogen, but no histopathological alterations.

Because of the recent interest in the mechanism of action of the antidiabetic sulfonylurea derivatives, the effect of chlorpropamide on the liver transaminase system was investigated, since an inhibition of transaminase reactions can lead to a decrease in the rate of neoglucogenesis. Serum transaminase values were determined in normal individuals, hepatic patients, and diabetics during therapy. Glutamate oxalacetic and glutamate pyruvate transaminase levels, as well as aldolase and lactic acid dehydrogenase activity, were investigated. It was observed that chlorpropamide inhibits serum transaminase levels as do other compounds, as shown by other investigators. It is suggested that by inhibiting transamination such derivatives decrease the rate of neoglucogenesis. This hypothesis would also explain the hepatotropic effects.

In our work at the Medical Clinic we have been particularly interested in determining minimal maintenance dosages. We have under study 35

patients 25 of whom received previous treatment and 10 of whom were diagnosed during the course of the study and received chlorpropamide as the initial therapeutic agent

Six of the patients were less than 25 years old and it was in this group that we observed our 2 failures. The other 4 patients are interesting since we did not observe an adequate response during the initial 2 weeks of treatment and therefore gave insulin in addition to chlorpropamide. We continued this combined therapy for 14 to 21 days after which we found surprisingly that the insulin dosage could be reduced and finally discontinued.

Our remaining 29 patients most of whom were 50 years of age or older and generally obese responded well to chlorpropamide. It is our practice to begin treatment with 1 gm per day for 3 or 4 days and then to reduce dosage to the lowest possible therapeutic level. We have found that most patients can be maintained in satisfactory balance with a dosage of 250 mg daily. However we have had success with as little as 100 mg daily and in an occasional patient with 100 mg every other day.

We have not observed any side effects from dosages of 1 gm daily or less. On higher dosage patients may experience nausea, discomfort in the epigastrium and a feeling of abdominal fullness. There was no indication of clinical toxic manifestations on the blood, skin, or nervous systems.

We were impressed with the relatively low minimal dosages of chlorpropamide that were fully effective in maintaining satisfactory sugar metabolism in patients with diabetes. Tolerance of dosages of 1 gm a day or less is excellent, probably more than that amount is not necessary since it may well represent the maximum therapeutic quantity. The average maintenance dosage is 250 mg daily and, not infrequently, even smaller doses are adequate.



# EXPERIENCES WITH CHLORPROPAMIDE, ESPECIALLY IN THE BRITTLE DIABETIC

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It has been shown that chlorpropamide is a sulfonylurea with antidiabetic action. Early in our clinical trial of this drug, we attempted to evaluate its control of the brittle or labile diabetic. We also attempted to determine whether the administration of very large doses would result in a more rapid control of the diabetes, and whether very large maintenance doses would control the brittle diabetic patient when average dosages failed to do so. Thus far, 34 patients with diabetes mellitus have been given chlorpropamide under our supervision. All of these patients were adhering to a restrictive diet of the American Diabetic Association type, 11 were taking, in conjunction with the diet more than 34 U of insulin daily in order to achieve control of their diabetes.

Of the 34 diabetic patients receiving chlorpropamide therapy, the drug was discontinued in 3 because of ineffectiveness of control, and in 5 others because of untoward effects. One patient was lost to our control, and 1 patient died.

## Initial Dosage

Twenty one patients were given an initial dosage of more than 1 gm of chlorpropamide daily. Of these, 12 achieved adequate control. Two patients received 4 gm as an initial dosage, neither achieved adequate control. Ten received 3 gm as an initial dosage, of these, 6 were inadequately controlled. Five patients received initial dosages of 2 gm, 3 of these were inadequately controlled (TABLE 1).

TABLE 1  
 CONTROL ACHIEVED WITH MORE THAN 20 GRAMS OF CHLORPROPAMIDE AS INITIAL DOSE

Initial dose (gm)	Number of patients	Control	
		Adequate	Inadequate
2.0	5	3	2
3.0	10	4	6
4.0	2	0	2

## Maintenance

Seven of our patients received maintenance dosages of more than 1 gm daily, of these, 3 had adequate control. Of 3 patients receiving more than 2 gm and as much as 4 gm daily for maintenance, none was controlled.

One of these patients received 4 gm daily for 4 days and achieved a blood level of chlorpropamide of more than 50 mg per cent. Although she had no toxic effects or symptoms from this drug, it was discontinued because of lack of control (TABLE 2).

TABLE 2  
MAINTENANCE DOSE (CHLORPROPAMIDE ONLY) AND RESULTS ACHIEVED

Maintenance dose (gm.)	Number of cases	Control	
		Adequate	Inadequate
0-25	1	1	
0.25-0.5	14	12	2
0.6-1.0	12	6	6
1	4	3	1
2-3	11	0	0
3	0	0	0
4	3	0	3

#### Control

Of 11 patients requiring a restrictive diet and more than 34 U of insulin daily for control of their diabetes, only 4 were able to continue chlorpropamide therapy for more than 4 weeks. The other 7 were returned to insulin because of inadequate control of these, 6 had disturbing side effects that, in themselves, would have been sufficient reason for discontinuing the drug. Three patients in whom control of the diabetes was lost on tolbutamide therapy were well controlled on chlorpropamide.

#### Side Effects

The number of side effects seemed to bear no direct relationship to the maintenance dosage of chlorpropamide. More side effects were noted in

TABLE 3  
CORRELATION OF DAILY DOSAGE OF CHLORPROPAMIDE WITH SIDE EFFECTS

Dosage (gm.)	Number of cases	Side effects			
		Skin	Gastrointestinal	CNS	Other
0-25	1				
0.25-0.5	14	2	5	6	
0.6-1.0	12		3	3	
1-2	7				1*
Total		2	8	9	1*

\* Decreased

patients receiving 0.5 gm daily than in those on higher daily dosages (TABLE 3). One patient, who had received large doses of chlorpropamide, died while receiving the drug. He suffered from arteriosclerotic and coronary artery heart disease in association with probable portal cirrhosis of the liver. Despite symptoms of anorexia and lethargy, the drug was continued until about thirty six hours before death. At no time was hypoglycemia present. Autopsy findings showed nonspecific changes of fatty metamorphosis of the liver, cerebral atrophy, an old healed myocardial infarction, and slight aortic atherosclerosis.

### *Conclusions*

In a small series of thirty four cases studied thus far we have found that Chlorpropamide was not effective in control of the brittle diabetic.

The effectiveness of chlorpropamide is not increased by large dosages, even as much as 4 gm daily.

The toxicity of this drug apparently increases with large doses, but some patients can tolerate large daily doses and high blood levels without ill effects.

High dosage of more than 1.0 gm daily was not associated with a higher incidence of side effects.

The drug is apparently effective in the mild diabetic.

Three patients not controlled by tolbutamide were controlled adequately by chlorpropamide.

One patient died while on large doses of chlorpropamide. The exact cause of death is unknown.

## A PRELIMINARY METABOLIC AND CLINICAL EVALUATION OF CHLORPROPAMIDE

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The metabolic effects and clinical efficacy of chlorpropamide, an oral hypoglycemic agent with potential therapeutic value, were evaluated in twenty three patients with diabetes mellitus. A preliminary report of this experience with chlorpropamide is presented here.

### *Materials and Methods*

During the course of these studies 10 patients with diabetes mellitus were selected for special study on the metabolic ward. Four of these patients, when placed under conditions of strict diet control similar to that previously recommended for them, did not demonstrate glycosuria and required no insulin. Accordingly trials of chlorpropamide therapy were not made with these 4 patients, and they were discharged from the hospital with diet therapy alone. The other 6 were considered suitable for the metabolic studies described below. Brief descriptions of these patients are included with the data obtained for each, as presented under *Results*.

The patients were maintained on constant diabetic diets designed to meet individual requirements, each daily diet being divided into 4 feedings. The patients were weighed daily, fluid intakes were recorded, but the subjects were permitted to drink water at liberty. Twenty-four hour outputs of urine were collected and preserved with 10 ml. of a 1:10 mixture of toluol and glacial acetic acid and refrigeration. Each daily urine volume was analyzed quantitatively for sugar content.<sup>1,2</sup>

The periods of study ranged from 18 to 62 days (average 39 days). Control periods of from 3 to 8 days preceded the administration of chlorpropamide in all patients. When it seemed permissible, insulin was withdrawn or reduced in dosage before the drug was given. The chlorpropamide\* was given orally, 1 gm. daily each morning before breakfast. One-gram doses of tolbutamide† were similarly given. Tolbutamide in daily doses greater than 1 gm. was administered in divided doses at mealtimes.

Fasting blood samples were obtained frequently for sugar determinations.<sup>1,2</sup> Fasting blood levels of chlorpropamide and of tolbutamide were determined serially in some patients.<sup>3</sup> For 3 patients, blood levels of chlorpropamide were determined several times within the 4-hour period immediately following a 1-gm. oral dose of the drug.

These studies included frequent urinalyses, complete blood counts, and tests of liver functions, including serum bilirubin concentration, alkaline phosphatase, cephalin cholesterol flocculation, and thymol turbidity. In

\* Provided by Chas. Pfizer & Co., Inc., Brooklyn, New York.

† Provided by The Upjohn Company, Kalamazoo, Mich.

some patients. Bromsulphalein excretion tests also were obtained before during and after the administration of the hypoglycemic agent. In addition the patients were observed closely for evidences of untoward reactions to chlorpropamide and for symptomatic hypoglycemia.

Chlorpropamide was also evaluated in 3 patients with diabetes, who were hospitalized on the general medical wards and in 14 patients attending the diabetes clinic as outpatients. These patients were selected on the basis of their likelihood to respond to an oral hypoglycemic agent for example patients with stable maturity onset type diabetes. The 3 hospitalized patients had not received insulin for at least 3 days before chlorpropamide was given. They were given 1 gm chlorpropamide daily for at least 6 days. The outpatients were instructed to discontinue insulin and to begin chlorpropamide on the same day. Initial dose of chlorpropamide for the outpatients was 1 gm daily for 1 week and 0.5 gm daily was prescribed thereafter. This therapy was continued for from 1 to 3 months (average 9 weeks). The patients visited the outpatient department weekly. No special effort was made to ensure more adequate adherence of the patients to their diets or to other diabetic instruction previously given. They were advised to continue their diabetic regimens as in the past. These patients were aware of course that a change in medication was made for example an injection of insulin was being replaced by an oral medication and in some cases, the frequency of required visits to the outpatient department was increased. Chlorpropamide was evaluated in the outpatients by fasting blood sugar determinations and fractional qualitative urine sugar determinations (Somogyi method). Blood sugar determinations were done almost daily in the hospitalized patients and usually weekly in the outpatients. In addition daily quantitative glycosuria was determined for the patients in the hospital. Blood chlorpropamide levels were determined at weekly intervals in some of the outpatients. Urinalyses, hemograms, blood urea nitrogen concentration and liver function tests were also done at intervals for these patients.

### Results

*Metabolic studies.* Since it was not possible to carry out identical protocols for the six patients studied on the metabolic ward the data for each of these patients are presented separately.

Patient J. M., a 42 year old Negro male diabetic for 1 year was admitted to the hospital because of diabetic acidosis. Of his own accord he had stopped taking insulin 6 months prior to admission. The acidosis was treated and he required 15 U. NPH insulin daily for 2 weeks preceding the metabolic study. The results of this study are presented in Figure 1. During the initial 7-day period of metabolic study this patient's diabetes was well controlled with a 2500 cal diet and 15 U. of NPH insulin daily. Insulin was then discontinued. During the 6 days without insulin glycosuria increased from less than 5 gm to about 30 gm daily and fasting blood sugar levels increased from 160 mg to 210 mg per cent. Then 1 gm chlorpropamide was given orally daily. In association with chlorpropamide therapy glycosuria and fasting blood sugar levels promptly decreased.

Twenty four hours after the initial 1 gm dose had been given fasting blood sugar level had decreased from 210 mg to 164 mg per cent and, after several days ranged between 110 and 132 mg per cent. Glycosuria was less than 5 gm daily and remained so even after the dose of chlorpropamide was decreased to 0.5 gm daily on the tenth day of therapy. Upon discharge from the hospital a similar diet and 0.5 gm chlorpropamide were prescribed for this patient who has been seen in the outpatient clinic at regular intervals and has maintained excellent control of diabetes under this regimen during

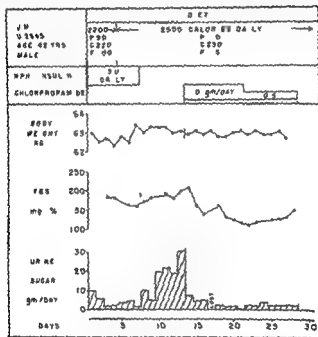


FIGURE 1

the 6-month period to date. The patient demonstrated a prompt and adequate hypoglycemic response to chlorpropamide administered orally and it replaced insulin in his diabetic regimen.

Patient A.M., a 60-year-old Negro female with stable, easily controlled diabetes of 10 years' duration, required 30 U of NPH insulin daily. This patient had a past history of repeated episodes of pancreatitis and it seemed probable that the diabetes was secondary to chronic pancreatitis. She was being treated in the hospital for pulmonary tuberculosis and received rifampin and para-aminosalicylate while this study was in progress. Insulin was discontinued 1 week before metabolic study was initiated. The findings in patient A.M. are presented in FIGURE 2. During the first 3 days of study this patient was given no insulin but was maintained on a constant 2000-cal

diabetic diet : Glycosuria increased from 68 gm to 94 gm daily, fasting blood sugar levels increased from 194 mg to 360 mg per cent, and the patient lost weight : Chlorpropamide, 1 gm daily, was then given orally After 48 hours, glycosuria had decreased to 54 gm daily, fasting blood sugar level had decreased to 228 mg per cent, and body weight was maintained Chlorpropamide was given for 6 days, following which fasting blood sugar levels

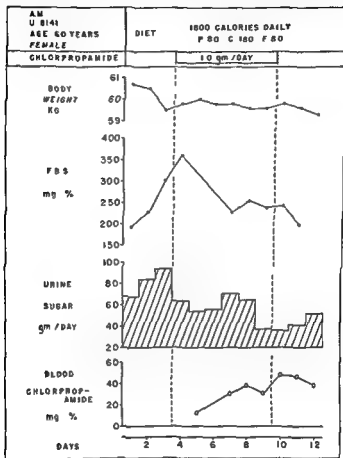


FIGURE 2

were about 200 mg per cent and glycosuria 35 to 40 gm daily. Although this patient demonstrated a hypoglycemic response to chlorpropamide, glycosuria was not decreased sufficiently to warrant considering diabetic control as excellent. Fasting blood levels of chlorpropamide were determined in this patient during and after the administration of the drug. These levels increased progressively during therapy to 54 mg per cent (FIGURES 2, 3).

Patient M L, a 62 year old Negro female, had stable diabetes of 12 years' duration that was not well controlled with 10 U NPH insulin daily. She

was admitted to hospital for regulation of diabetes. This patient also had arteriosclerotic heart disease and took digitalis daily. The initial 5 day control regimen for M. L. (FIGURE 4) consisted of a 2000 cal diet and 10 U of NPH insulin daily. During this period fasting blood sugar levels ranged

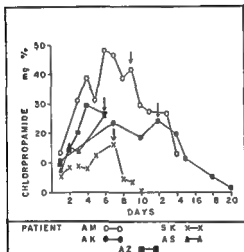


FIGURE 3 Blood levels after oral administration of 1 gm per day of chlorpropamide. Arrows indicate the days on which chlorpropamide was discontinued.

eventual replacement of insulin by this agent. The apparent slow response to chlorpropamide makes this study somewhat difficult to interpret but since it had been known that this patient had diabetes for 12 years and had required insulin during this time, this case is considered a positive response to chlorpropamide.

Patient A. K. (FIGURE 5) a white female 63 years of age was found to have diabetes following a recent admission to the hospital for thrombophlebitis of the left lower leg. She also demonstrated senile dementia but was able to cooperate in the adequate collection of urine specimens. Prior to the initiation of study she had persisting glycosuria of about 20 gm daily although she was given a prescribed diet and 40 U of NPH insulin daily.



This patient had about 25 gm glycosuria daily, and fasting blood sugar levels of 280 mg per cent during the 2 initial days of observation on the metabolic ward while she was consuming a 2000 cal diet and receiving 40 U of NPH insulin daily. Two grams of tolbutamide, given orally, in addition to the insulin, produced a prompt decrease in glycosuria to less than 5 gm daily and in fasting blood sugar to 170 mg per cent, this was apparent after the first day of therapy. Discontinuance of insulin was followed by transitory increases in urine and blood sugar levels, but control of diabetes continued

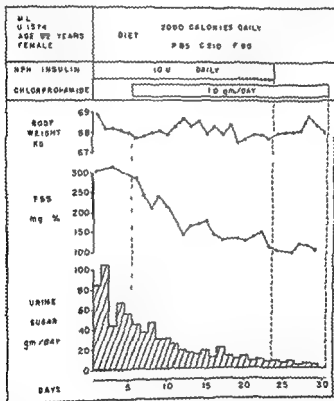


FIGURE 4

to be adequate. After 11 days of tolbutamide administration, this agent was discontinued following which glycosuria and hyperglycemia increased, reaching values of 30 gm daily and 250 to 280 mg per cent after 8 days. One gram of chlorpropamide was given daily for the next 13 days. Chlorpropamide was discontinued after 13 days. Glycosuria was not decreased after 13 days as with tolbutamide. After chlor-

propamide was not decreased after 13 days as with tolbutamide. After chlor-

propamide was discontinued glycosuria and hyperglycemia increased during a 9-day control period. During a final period of study 1 gm of tolbutamide was given daily. As in the earlier period of tolbutamide administration the effect of this agent on blood sugar and glycosuria was prompt. The urine sugar excretion decreased from about 30 gm daily to less than 10 gm on the first day this drug was used. Maximal blood sugar response was noted 48 hours after tolbutamide therapy was started by which time levels

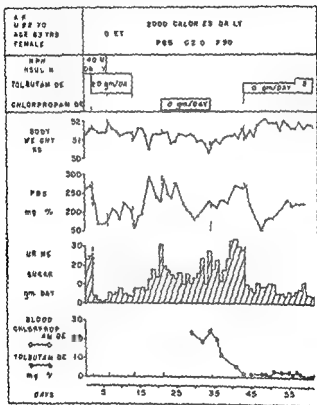


FIGURE 5

had decreased from about 265 mg per cent to 154 mg per cent. Blood sugar then increased gradually to approximately 230 mg per cent at which level it became stable. Glycosuria averaged less than 10 gm daily. Increasing the dose of tolbutamide from 1.0 to 1.5 gm had no appreciable effect on blood or urine sugar levels.

Serial fasting blood levels of chlorpropamide and tolbutamide were done for this patient (Figure 5). During the last 6 days of chlorpropamide therapy blood levels of this agent ranged from 18 to 25 mg per cent. After

chlorpropamide was discontinued. The blood glucose was approximately 7 mmol/L.

Only small amounts of tolbutamide were

samples obtained during the administration of this agent.

Both chlorpropamide and tolbutamide demonstrated hypoglycemic and aglycosuric effects in patient A. K. The response to tolbutamide was more prompt and more striking than the response to chlorpropamide.

Two additional patients (E. H. and S. K.) who were studied on the metabolic ward for thirty six and sixty days, respectively, failed to demonstrate any significant responses to the oral administration of chlorpropamide or of tolbutamide. Brief résumés of these patients follow.

Patient E. H., a 46 year old man, had very labile diabetes. He had experienced frequent episodes of acidosis and hypoglycemia, many of which required hospitalization during the 10 years since the diagnosis of diabetes mellitus was made. His usual daily insulin dosage was 40 U of PZI insulin each morning and 30 to 50 U of regular insulin during the day. He was not well controlled despite many efforts in the hospital to accomplish an adequate regimen. His basal control regimen consisted of a 1900 cal diet and insulin (as indicated above). Following a 12 day control period, he was given, in addition, 1 gm chlorpropamide daily for 22 days. This period of chlorpropamide administration was followed by the basal regimen for 14 days. Then 2 gm tolbutamide was given orally each day for 13 days. There was no effect attributable to either of these hypoglycemic agents.

Patient S. K., a lean man 47 years of age, was admitted to hospital in 1953. He responded promptly to insulin therapy and was well controlled. In 1954, he was transferred from a general medical ward to the metabolic ward.

glycemia or urinary sugar excretion. Following a 3 day control period without insulin, tolbutamide was given orally (1 gm, 3 gm, and 4 gm orally daily for 3 successive periods of 3 days each). Tolbutamide was also without effect.

101 S. K. (continued)  
6 to 17 mg per cent. After tolbutamide had been given for 9 days in dosages increasing from 1 gm to 4 gm daily, 14 mg per cent of tolbutamide was detectable in fasting blood specimens. The failures of response in this patient could not therefore be attributable to a failure of absorption of the hypoglycemic agents. In S. K., approximately 3 days were required for

the complete disappearance of chlorpropamide from the blood after cessation of therapy

**Chlorpropamide blood levels** In some patients, chlorpropamide blood levels were determined at intervals during the 4 hour period following a 1 gm oral dose. These data are presented in FIGURE 6. The average blood level was about 9 mg per cent after 1 hour, and 12 mg per cent 4 hours after its ingestion, indicating prompt absorption of this agent.

The serial chlorpropamide blood levels obtained during the studies of patients on the metabolic ward are presented compositely in FIGURE 3. The patients received 1 gram of chlorpropamide daily for the time indicated

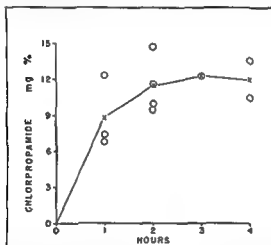


FIGURE 3 Blood levels after oral administration of 1 gm chlorpropamide. Curve from x to x indicates the average value for each interval following chlorpropamide administration.

on the chart. Blood levels of the drug were cumulative at this dosage. This relatively small group of patients demonstrated considerable variation in blood level attained with comparable doses of the drug. The rates of disappearance of chlorpropamide from the blood after therapy varied from 3 to 7 days in these patients.

The blood levels of chlorpropamide determined at weekly intervals in some of the patients attending the outpatient clinic are presented in TABLE 1. Although these data are admittedly meager, they suggest that maintenance doses of 0.5 gm daily of chlorpropamide may be adequate to maintain blood

60 year old female (newly discovered diabetic) responded adequately to chlorpropamide, in a dosage of 1 gm daily for 6 days with a decrease in fasting blood sugar to levels below 150 mg per cent, she became essentially

aglycosuric. Continued on a maintenance dose of 0.5 gm daily she has been observed in the outpatient clinic for 2 months during which time diabetic control has been sustained. The other 2 patients of this group (a 70 year old female and a 46 year old male) each of whom required 20 U of NPH insulin for control of diabetes did not respond adequately to 1 gm chlorpropamide given daily for 1 week; they were returned to insulin therapy.

The fourteen outpatients selected for study ranged in age from thirty to eighty three years with an average age of sixty one years. Diabetes had been established for periods of from six months to twenty eight years. The average duration of diabetes was ten years. In all patients the diabetes was the stable maturity onset type.

TABLE 1  
CHLORPROPAMIDE BLOOD LEVELS IN DIABETIC OUTPATIENTS

Weeks of therapy	Daily dose (gm)	No. of patients	Level mg per cent	
			Range	Average
1	1.0	3	30-34	32
3	0.5	6	14-33	23
5	0.5	3	13-24	18
7	0.5	2	18-20	19
9	0.5	2	20-23	22

Three of the patients had been treated with diet therapy only; the remainder had been treated with diet and from 10 to 35 U insulin. The average insulin dose was 25 U daily.

All patients were given similar doses of chlorpropamide: 1 gm per day for the first week followed by one half gm per day as a maintenance dose thereafter.

An adequate clinical response to this drug was established as one exhibiting aglycosuric fractional urines, lack of subjective symptoms, maintenance of body weight, and reduction in fasting blood sugars—preferably below 150 mg/100 cc.

In 12 patients the diabetes was adequately controlled with a single daily dose of chlorpropamide as judged by the above criteria. At present these patients continue to be treated with diet and chlorpropamide therapy. Seven showed an average reduction in the fasting blood sugar of 105 mg per cent from previous levels and continued to be maintained at levels below 150 mg per cent. Seven others showed no appreciable change in fasting blood sugar levels; however, most of them had been well controlled on their previous therapy.

One previously uncontrolled patient was controlled with 1.5 U c.c.i.

and was returned to insulin therapy. This patient was the only one to express subjective symptoms to chlorpropamide therapy. She complained of epigastric distress during treatment, which disappeared when chlorpropamide was discontinued, she was being treated simultaneously for a chronic duodenal ulcer.

The other patient, a 45 year old male with mild diabetes of 3 years' duration, previously controlled with 25 U of insulin, did not respond adequately after 4 weeks of treatment with chlorpropamide, insulin therapy was resumed.

Chlorpropamide appeared successful in controlling the diabetes in 12 of 14 of this group of selected outpatients, most of whom were in the older age group with stable diabetes previously treated with diet therapy alone or in conjunction with moderate doses of insulin. Control has been maintained for as long as 6 months.

**Toxicity** With the exception of the patient mentioned above, who experienced epigastric distress, there were no subjective symptoms of toxicity nor were there laboratory evidences of disturbances of renal, hepatic, or hematological function in any of the patients studied. Symptomatic hypoglycemia did not occur.

### Discussion

Chlorpropamide was an effective oral hypoglycemic agent when administered orally to most of the patients (with diabetes mellitus) studied. As with other oral hypoglycemic agents, patients with stable, maturity-onset type diabetes demonstrated more adequate responses than relatively young patients with labile diabetes. There were exceptions to this, however, in that two of the outpatients studied, who were judged to fulfill the accepted indications for therapy with an oral agent, failed to respond to chlorpropamide. Furthermore, the patterns of response to chlorpropamide, as evaluated in the six patients studied on the metabolic ward, were quite variable. One patient demonstrated a prompt and adequate response (J. M., FIGURE 1), another, a delayed response (M. L., FIGURE 4); others showed significant but inadequate responses (A. M., FIGURE 2, A. K., FIGURE 5). The reasons for this variation are not apparent; they did not relate to the blood level of chlorpropamide. Although blood levels varied considerably among patients given similar doses of the drug (FIGURE 3), blood level did not correlate with the adequacy of diabetic control attained with chlorpropamide.

Four patients who either failed to respond or demonstrated inadequate responses to chlorpropamide were subsequently given tolbutamide. Three of these also failed to demonstrate responses to tolbutamide (E. H., S. K., A. M.). A fourth patient (A. K., FIGURE 5) demonstrated more prompt and pronounced effects on blood and urine sugar when she was given tolbutamide than when she was given chlorpropamide in comparable dosage.

Studies of the level of chlorpropamide in the blood following its oral administration indicated that it is promptly absorbed and slowly excreted. Three to seven days were required after cessation of therapy before the drug disappeared from the blood. In this respect it differs from tolbutamide, which is promptly absorbed and more rapidly removed from the blood. With 1 gm

daily doses of chlorpropamide blood levels of the drug progressively increased in some patients. Those who demonstrated adequate responses to 1.0 gm of chlorpropamide given daily for about 1 week subsequently sustained adequate control of diabetes with a continuing dose of 0.5 gm daily. Presumably, it is possible that

doses less than 0.5 gm daily might have been satisfactory for maintenance in some patients. On the other hand, some patients might have demonstrated more adequate responses had larger doses been employed (S. A. FIGURE 3). In the studies reported here, no effort was directed to establish either the minimally effective dose of chlorpropamide or the maximum safe dose.

An important consideration in the acceptance of a drug for clinical use is its freedom from untoward effects. Although no serious untoward symptoms or laboratory findings resulted from the administration of chlorpropamide to the twenty-three patients studied, conclusions regarding toxicity in man must be based on a much larger experience than is reported here.

### Summary

The clinical and metabolic effects of chlorpropamide administered orally were evaluated in 23 patients with diabetes mellitus. Six of these were studied under carefully controlled conditions on a metabolic ward. The other 17 were observed on the general medical wards (3 patients) or in the outpatient clinic (14 patients).

Chlorpropamide given orally (1 gm daily) decreased glycosuria and fasting blood sugar levels in 4 of the 6 patients studied on the metabolic ward. Adequate clinical responses were obtained in 2 stable diabetics for whom insulin was completely replaced. Inadequate responses were found in 2 other stable diabetics who had required 40 U of NPH insulin daily. One of the patients who was inadequately controlled with chlorpropamide demonstrated more prompt and adequate decreases in glycosuria following identical doses of tolbutamide. The other 2 patients (a lean 47 year old man with newly discovered diabetes and a 46 year old man with very labile diabetes)

promptly, returned to normal blood sugar levels. In the 17 patients who were followed in the outpatient clinic, the blood following cessation of therapy. Blood levels and also with the same dose (1 gm daily) of chlorpropamide demonstrated considerable variation in the patients studied.

In 17 patients who were followed for 6 months with a single daily oral dose of chlorpropamide (0.5 gm daily for 1 week, and then 0.5 gm daily). With this dosage schedule, chlorpropamide blood levels of about 20 mg per cent were maintained.

With the exception of epigastric distress in one patient, there were no

untoward clinical manifestations attributable to chlorpropamide. Symptomatic hypoglycemia was not observed. Serial urinalyses, hemograms, and tests of hepatic and renal functions were not altered during the periods of study.

#### *Acknowledgment*

The chlorpropamide and tolbutamide blood levels were determined by arrangement with Max Miller, Western Reserve University School of Medicine. These determinations were done in his laboratories by Naomi F. Nickerson. The authors are grateful for this contribution to the study.

#### *References*

1. NELSON N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J Biol Chem* 153: 375.
2. SOMOGYI M. 1952. Notes on sugar determination. *J Biol Chem* 195: 19.
3. TOOLAN T J & R L WAGNER JR. 1959. The physical properties of chlorpropamide and its determination in human serum. *Ann N Y Acad Sci* 74(J): 449.



# CHLORPROPAMIDE A NEW SULFONYLUREA STUDIED IN THE TREATMENT OF THIRTY DIABETIC PATIENTS

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A few years ago insulin was the only drug available for the control of diabetes. In 1955, German authors<sup>1, 2</sup> reported the results of the treatment of diabetic patients with carbutamide, an oral sulfa drug with a marked hypoglycemic effect. Shortly thereafter a new compound known as tolbutamide, closely related to the first, was also employed.<sup>3</sup> Loubatieres reviewed in a complete and concise presentation the history and development of these compounds.<sup>4</sup>

diabetics or in thin mature individuals who have received insulin for several years

Chlorpropamide, a recently developed oral drug closely related to tolbutamide has also shown a hypoglycemic effect in animal studies. The purpose of this paper is to report our experience during the last eight months in administering this compound to diabetic patients.

## Methods

20 — — (15 — 10 and 26 males) observed

20 as moderately severe and 6 as mild. The disease had started from a few months to 20 years before but in most cases, it had existed 1 to 8 years. There was 1 case of diabetes (No. 29) due to tumoral invasion of the pancreas.

Twenty four patients were on insulin therapy or had received this drug for some time. Twenty four were taking or had taken oral drugs: 12, carbutamide and 12 tolbutamide. Five had not had any previous therapy.

TABLE 1 contains all pertinent data concerning each of the patients studied.

A careful physical examination was performed. blood sugar<sup>5</sup> was determined at least twice a week, and urine was examined for sugar four times a day before meals. The following tests were also done: complete urinalysis, blood count and differential, blood urea, creatinine and bilirubin, thymol turbidity, colloidal gold, cephalin cholesterol flocculation, serology and sedimentation rate.

The patients were on a standard diabetic diet. Oral antidiabetics were

discontinued for 1 or 2 weeks and insulin injections were discontinued for 48 hours. A chlorpropamide tolerance test was then performed. A fasting venous blood sample having been obtained 3 gm of the drug was given orally. 4 more hourly blood specimens were then taken. Blood sugar was determined according to Fohn's micromethod.

TABLE 1  
DATA ON THIRTY PATIENTS TREATED WITH CHLORPROPAMIDE

Case no.	Age	Sex	Weight per cent	Severity of diabetes*	Duration years	Insulin	Carbutamide	Tolbutamide	Chlorpropamide to tolerance test			Blood sugar reduction percentages	Average doses per day	Length of treatment days	Result
									First hour	Second hour	Fourth hour				
1	56	M	-5	+	1	0	0	0					0 125	90	B
2	42	F	+38	++	4	+	0	+	190	145	90	43	1	7	C
3	51	F	+38	++	7	+	+	+	185	185	134	28	1 250	10	B†
4	55	M	+12	++	7	+	0	0	178	155	123	31	0 750	98	E
5	49	M	+22	++	6	+	+	■	95	100	83	8	0 500	180	E
6	53	M	+31	+	1	0	0	0	356	270	258	18	0 250	150	B
7	34	M	+15	+	4	+	0	0	107	99	86	20	0 250	105	F
8	17	M	-6	+	1	+	0	0	80	50	30	63	1	105	E
9	45	M	-28	+++	12	+	0	+	250	280	290	+			
10	■	M	-5	++	■	+	+	■	150	81	57	56	0 250	197	E
11	40	M	+19	++	4	+	+	0					0 875	114	E
12	21	M	13	+++	4	+	0	0	215	215	215	0			
13	65	M	+11	++	8	+	0	+	180	160	143	18	1 500	67	F
14	46	F	+5	++	3	+	0	+	160	150	129	20	1	■	G
15	65	M	-14	++	8	+	0	+	300	320	295	1			
16	31	M	+8	+	1	0	0	0	228	198	170	26	0 250	111	E
17	53	M	+16	++	4	+	+	+	160	125	93	43	0 500	56	E
18	70	M	+19	++	10	+	+	0					0 250	191	E
19	37	M	+7	++	2	+	0	+	142	85	95	33	0 250	185	E
20	67	M	-20	++	8	+	+	0					0 125	200	E
21	57	M	-14	++	1	+	+	+	222	216	178	20	2	30	E
22	32	F	+19	++	10	+	+	■	190	190	120	37	1 500	213	G
23	46	M	+32	++	9	+	+	■	128	120	110	14	0 750	200	E
24	42	M	-1	+++	5	+	0	+	225	240	230	+			
25	35	M	-11	+	1	0	0	0	170	152	112	34	0 500	119	E
26	65	M	-24	++	20	+	+	+	160	107	86	49	1 250	70	G
27	54	M	-5	+++	5	+	0	0	175	210	215	+			
28	52	M	+18	++	1	0	0	0	194	170	116	40	0 500	10	E
29	■	M	■	■	■	+	+	0	180	155	140	22	2 250	22	G
30	49	M	+25	++	9	+	0	+	230	187	160	31	1	8	F

\* Key: + mild ++ moderately severe +++ severe

† Treatment of scint used because of intolerance

‡ Key: E excellent G good F fair B bad

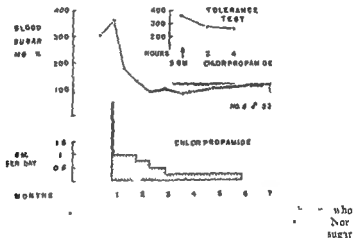
This initial test dose was followed in most of the cases by 1 gm daily taken one half after breakfast and one half after dinner. This dosage was gradually reduced according to the blood sugar level. Control was continued every 2 or 3 weeks at the outpatient clinic. Sugar was checked in blood and

urine each time Periodically, hepatic, renal, and hematological tests also were performed

### Results

Twenty four of 30 patients studied have been adequately controlled with chlorpropamide and a standard diabetic diet for as long as 7 months. Fourteen had a blood sugar reduction of 25 per cent or more when the tolerance test was performed FIGURE 1 illustrates a response typical of patients in the group Four patients had reductions of 20 per cent, and 1 each of 18, 14, and 8 per cent The average maintenance dose was 600 mg per day, varying between 125 and 2000 mg

One patient (No 21), who had a 20 per cent blood sugar reduction at the tolerance test, could not be controlled with chlorpropamide alone and



required insulin. Nevertheless after several days of combined therapy, it was found that insulin could be discontinued leaving the patient on the oral drug alone (FIGURE 2). It is interesting to note that this patient had also had a 21 per cent blood sugar reduction when 3 gm of tolbutamide was given; however, control could not be achieved with this drug alone.

Another patient (No 13) had an 18 per cent sugar reduction, but only a partial control was obtained. Symptoms disappeared, but blood sugar level was brought no lower than 150 mg per cent.

The tolerance test was negative in five patients (Nos 9, 12, 15, 24, and 27), showing either no reduction of the initial blood sugar level or a rise. All had shown the same lack of response to carbutamide and tolbutamide and all had a diabetes of long duration and had been on insulin therapy for more than four years.

Chlorpropamide, 1.5 gm daily, was given in conjunction with insulin in 3 of these cases. A mild favorable effect was demonstrated in 2 (Nos 12 and 15) and none in the third (No 27).

Chlorpropamide was tolerated very well by most of the patients. There were two (Nos 13 and 20) who complained of mild dizziness (like drunkenness) not related to hypoglycemia and lasting a few days. During this time they were able to continue their work. The discomfort gradually disappeared and the treatment was not discontinued.

Only 1 patient (No 3) showed marked intolerance. She had had a 28 per cent blood sugar reduction on the initial test and did not complain of any immediate discomfort at that time. Nevertheless she developed nausea

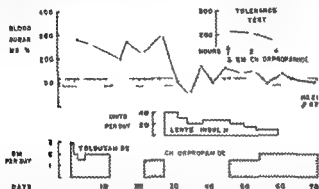


FIGURE 2 Chart of a 57 year-old Negro male (patient No. 21) who had a 21 per cent reduction in blood sugar during the tolbutamide tolerance test and a 20 per cent reduction with chlorpropamide. Blood sugar could not be brought to normal after 12 days treatment with tolbutamide (2 gm per day). Chlorpropamide (1.5 gm per day) was given for 8 days without success. Forty U per day of lente insulin was then injected; blood sugar was lowered rapidly to normal levels. After 17 days of good carbohydrate metabolism chlorpropamide was resumed and insulin was gradually reduced and finally discontinued.

vomiting, epigastric burn, diarrhea, dizziness, and headache after a few days of treatment at a dosage of 1.5 gm daily. Symptoms stopped when the drug was discontinued but reappeared each time an attempt was made to resume the treatment. She had tolerated tolbutamide well but the drug did not control her diabetes.

In none of the thirty cases were skin reactions or hematological, renal, or hepatic changes observed.

No particular reaction was noticed in four patients (Nos 5, 19, 20, and 23) who had pulmonary tuberculosis. One with a partial gastrectomy performed 3 years before because of a perforated duodenal ulcer had an excellent evolution. He is taking 250 mg every other day and has been observed for 200 days.

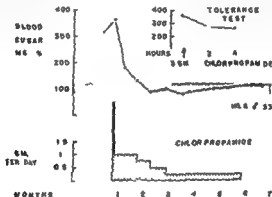
No changes in the blood pressure level occurred. Two patients (Nos 6 and 17) with high blood pressure and normal renal function tests had to continue taking hypotensive drugs. Two others with arterio-sclerosis and high blood pressure did not show any change (Nos 10 and 26). One (No 10) who had renal insufficiency (urea 100 mg per cent) was maintained on a low protein diet during 197 days of therapy. In addition to an excellent carbo-

urine each time. Periodically hepatic, renal, and hematological tests also were performed.

### Results

Twenty four of 30 patients studied have been adequately controlled with chlorpropamide and a standard diabetic diet for as long as 7 months. Fourteen had a blood sugar reduction of 25 per cent or more when the tolerance test was performed. FIGURE 1 illustrates a response typical of patients in the group. Four patients had reductions of 20 per cent, and 1 each of 18, 14, and 11 per cent. The average maintenance dose was 600 mg per day, varying between 125 and 2000 mg.

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Chlorpropamide was tolerated very well by most of the patients. There were two (Nos 13 and 20) who complained of mild dizziness (like drunkenness) not related to hypoglycemia and lasting a few days. During this time they were able to continue their work. The discomfort gradually disappeared, and the treatment was not discontinued.

Only 1 patient (No 3) showed marked intolerance. She had had a 28 per cent blood sugar reduction on the initial test and did not complain of any immediate discomfort at that time. Nevertheless she developed nausea,

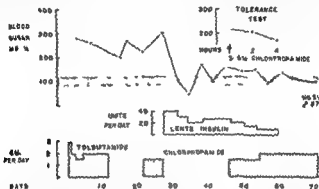


FIGURE 2 Chart of a 57 year-old Negro male (patient No 21) who had a 21 per cent reduction in blood sugar during the tolbutamide tolerance test and a 20 per cent reduction with chlorpropamide. Blood sugar could not be brought to normal after 12 days treatment with tolbutamide (2 gm per day). Chlorpropamide (1.5 gm per day) was given for 8 days without success. Forty U per day of lente insulin was then injected. Blood sugar was lowered rapidly to normal levels. After 17 days of good carbohydrate metabolism, chlorpropamide was resumed and insulin was gradually reduced and finally discontinued.

vomiting, epigastric burn, diarrhea, dizziness and headache after a few days of treatment at a dosage of 1.5 gm daily. Symptoms stopped when the drug was discontinued but reappeared each time an attempt was made to resume the treatment. She had tolerated tolbutamide well, but the drug did not control her diabetes.

In none of the thirty cases were skin reactions or hematological, renal, or hepatic changes observed.

No particular reaction was noticed in four patients (Nos 5, 19, 20, and 23) who had pulmonary tuberculosis. One, with a partial gastrectomy performed 3 years before because of a perforated duodenal ulcer, had an excellent evolution. He is taking 250 mg every other day and has been observed for 200 days.

No changes in the blood pressure level occurred. Two patients (Nos 6 and 17) with high blood pressure and normal renal function tests had to continue taking hypotensive drugs. Two others with arteriosclerosis and high blood pressure did not show any change (Nos 10 and 26). One (No 10), who had renal insufficiency (urea, 100 mg per cent) was maintained on a low protein diet during 197 days of therapy. In addition to an excellent carbo-

hydrate balance, a slight decrease in blood urea level was achieved (urea, 80 mg per cent). This patient is now taking 250 mg per day.

### Discussion

Chlorpropamide seems to have the same mode of action as carbutamide and tolbutamide, but it seems to be more powerful most patients requiring a lower dosage.

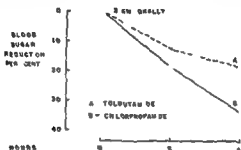


FIGURE 3 Comparative average blood sugar reduction (percentage) in nine patients. Reduction was 17.5 per cent for tolbutamide and 35.3 per cent for chlorpropamide.

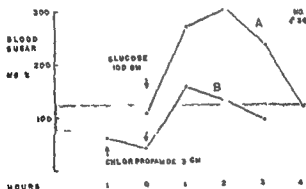


FIGURE 4 An abnormal oral 100 gm glucose tolerance test (patient No. 1) became almost normal when repeated 1 hour after administration of 3 gm of chlorpropamide.

When a 4 hour tolbutamide tolerance test was performed on 9 patients, an average blood sugar reduction of 17.5 per cent was found. A chlorpropamide

propamide previously had not been balanced with other oral drugs alone, requiring insulin also.

An abnormal glucose tolerance test in 1 patient (No. 1) showed an almost

normal reaction when repeated 1 hour after the patient had taken 3 gm of chlorpropamide (FIGURE 4)

It is interesting to note that a seventeen year old patient (No 8) has been very well controlled with this drug

### *Summary*

The activity of chlorpropamide in 30 diabetic patients formerly treated with insulin carbutamide, or tolbutamide has been studied. Venous blood sugar levels during the 4 hours following oral administration of 3 gm of chlorpropamide were previously determined. The drug was administered to 27 of these patients for as long as 7 months. The percentage of blood sugar reduction obtained in the initial test is related to the results of the treatment. The curves plotted from these data are compared with similar tests that used tolbutamide.

The drug was well tolerated. Its mode of action seems to be similar to that of previously used sulfa drugs. Nevertheless chlorpropamide seems to be more powerful and, in some cases, more effective, a lower dosage was required by most patients. It was possible to keep in good balance some cases that had not responded adequately to other oral drugs. No skin reactions and no hematological, renal, or hepatic changes were observed.

### *Acknowledgment*

The authors are indebted to German Stiglich and Luis Espejo for their cooperation. The valuable technical assistance of Laura Garcia and Albuina Muñoz is acknowledged. The chlorpropamide used was furnished by Chas Pfizer & Co, Inc, Brooklyn, N Y.

### *References*

- 1 BERTRAM F E BENDFELT & H OTTO 1955 An active peroral antidiabetic (BZ 55) Deut med Wochschr 80 1455
- 2 FRANKE H & J FUCHS 1955 A new antidiabetic principle Deut med Wochschr 80 1449
- 3 ACHELIS J D & K HARDEBECK 1955 A new hypoglycemic substance Deut med Wochschr 80 1452
- 4 LOUBATIÈRES, A 1957 The hypoglycemic sulfonamides Ann N Y Acad Sci 71(1) 4
- 5 GOLIN O 1928 A new blood sugar method J Biol Chem 77 421



# PRELIMINARY REPORT ON THE TREATMENT OF DIABETES MELLITUS WITH CHLORPROPAMIDE\*

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Chlorpropamide was made available to us early in July 1958. To initiate the investigation, 113 unselected diabetic patients were chosen. Twenty-five of these were rejected because they showed satisfactory control with diet alone, and 6 were considered not suitable for exposure to chlorpropamide because of severe metabolic nephropathy. In 3 cases treatment was suspended because of intercurrent conditions not related to the use of the drug under investigation and, in 27 cases, the study was discontinued for non-medical reasons. Of the original group, 52 were left to be considered in this study which, because of the small number of cases that have been studied thus far and the shortness of the observation periods (5 to 36 days), should be regarded as a preliminary report.

## *Material and Methods*

All patients were treated on an ambulatory basis, they and their relatives received careful instructions regarding such aspects as diet and the performance of urine test for sugar. On the days the blood sugar was determined the patients were admitted to the outpatient dining room of the Monte Carmelo Medical Clinic, where the meals were served. Every patient under study was submitted to the following controls: (1) medical consultation at least once a week, (2) determination of venous blood sugar (Folin Wu method), while fasting and between meals, (3) routine urinalysis, and (4) hematological studies (red and white cells, platelet counts, hemoglobin and hematocrit determinations, and differential white counts), as well as blood urea nitrogen determinations.

Generally speaking, the diets prescribed had the following approximate composition for a 24 hour period: 200 to 250 gm of carbohydrates, 1 to 1.5 gm of protein per kilogram of body weight, and fat to complete the caloric requirements. Because of food habits in Venezuela, the diet contained liberal amounts of foods rich in easily available carbohydrates (rice, bread, pastry, cornbread, potatoes, and tubers such as yucca and ocumo). For the same reason the distribution of the carbohydrates of the diet was, in general, as follows: 20 per cent at breakfast, 5 per cent at mid morning, 40 per cent at lunch, 10 per cent at 4 P.M., and 25 per cent at dinner. In those patients in which it was advisable not to omit insulin completely, only the regular type of this hormone was kept in use, administered to the patients 2 to 3 times a day according to the amount of glycosuria present before breakfast, lunch, or dinner (or both the latter). Regarding chlorpropamide, in most cases we used rather small doses, trying to determine the smallest

\* The study reported here was supported in part by a grant from the Pfizer Corporation of Venezuela, supplier of the chlorpropamide used.

active one necessary to control the patients receiving it (TABLE 1). In a few cases with severe diabetes we prescribed larger doses but in no instance was any dose more than 500 mg. 3 times a day. We do not know whether the number of patients showing satisfactory response would have been larger if we had used larger doses or if we had given the drug for a longer period of time or both. It is to be noted that in 3 cases it seemed that a better action of the drug upon the blood sugar was obtained if chlorpropamide was given 1 hour before meals. It should be noted at this point that preliminary investigation in fasting diabetics showed that the action of the drug was more consistent 3 to 4 hours after it had been administered. Owing to the short time available to complete this work it was decided to stop treatment after

TABLE 1  
SCHEDULE OF TREATMENT WITH CHLORPROPAMIDE

Day of treatment	Milli grams		
	Breakfast	Lunch	Dinner
First	250	250	250
Second	250	125	250
Third	250	125	125
Fourth and beyond	125	125	125

8 to 10 days in cases with poor response. We feel now that those patients who respond satisfactorily will do so after 5 to 7 days of treatment.

#### Analysis of Results

The patients were grouped arbitrarily according to their responses as follows (TABLE 2).

Group A is composed of patients with excellent responses (no insulin needed) and includes those in good clinical condition who showed no glycosuria and had blood sugar values between normal limits (fasting and after meals).

Group B includes patients with good responses (no insulin needed) in whom fasting blood sugar is not more than 150 mg. per cent and not more than 200 mg. per cent 2 hours after each meal. Slight glycosuria will exist but no acetoneuria.

Group C is made up of those showing a fair response (with or without insulin) and a fasting blood sugar between 150 and 200 mg. per cent. Blood sugar after meals is between 200 and 250 mg. per cent with moderate glycosuria.

Group D includes patients showing a poor response (with or without insulin) and a fasting blood sugar of more than 200 mg. per cent and more than 250 mg. per cent after meals. Permanent and accentuated glycosuria is present with or without acetone in the urine.

TABLE 2  
VENOUS BLOOD SUGAR IN DIABETICS TREATED WITH CHLORPROPAMIDE

Type of response	Group average, mg per cent*			
	Fasting	2 hours after breakfast	2 hours after lunch	2 hours after dinner
Excellent (A)	115	118	136	125
Good (B)	140	156	189	185
Fair (C)	163	190	240	238
Poor (D)	225	260	320	296

\* Figures were obtained by blood sampling each patient at least twice at each time but on different days

TABLE 3  
RESULTS OBTAINED IN 52 DIABETIC PATIENTS TREATED WITH CHLORPROPAMIDE\*

Response	No of cases	Percentages
Excellent (A)	10	19.2
Good (B)	18	34.6
Fair (C)	4	7.6
Poor (D)	20	38.4
Satisfactory results (group A plus group B)	28	53.8

\* Age at onset of diabetes all ages

TABLE 4  
RESULTS OBTAINED IN 30 DIABETIC PATIENTS TREATED WITH CHLORPROPAMIDE\*

Response	No of cases	Percentages
Excellent (A)	9	30
Good (B)	16	53.3
Fair (C)	2	6.6
Poor (D)	3	10
Satisfactory results (group A plus group B)	25	83.3

\* Age at onset 40 years or more

According to this classification (TABLE 3) we obtained satisfactory results in 53.8 per cent of the cases (groups A and B), and unsatisfactory responses in 46.0 per cent of the series (groups C and D). However, it is noteworthy that, in the group with poor response (20 cases, or 38.4 per cent of the group in which no satisfactory results were obtained), there were 5 patients who had developed diabetes before 10 years of age, 6 in whom the diabetes had had an onset between 11 and 20 years of age, and 6 in whom the onset age

TABLE 5  
RESULTS OBTAINED IN 22 DIABETIC PATIENTS TREATED WITH CHLORPROPAMIDE\*

Response	No. of cases	Percentages
Excellent (A)	1	4.5
Good (B)	2	9.0
Fair (C)	2	9.0
Poor (D)	17	77.2
Satisfactory results (group A plus group B)	3	13.5

\* Age at onset less than 40 years

TABLE 6  
DATA ON 28 DIABETIC PATIENTS SHOWING EXCELLENT\* AND GOOD† RESPONSE TO CHLORPROPAMIDE

Case No	Age at onset of diabetes (years)	Duration of diabetes (years)	Age (years)	Insulin U 24 hours	Body type (constitution)
1	30	3	33	0	Athletic
2	53	6	59	0	Asthenic
3	45	4	49	0	Pyknic
4	45	9	54	0	Pyknic
5	46	4	50	10	Pyknic
6	50	3	53	0	Pyknic
7	40	8	48	0	Pyknic
8	50	13	63	16	Pyknic
9	37	5	62	0	Pyknic
10	43	8	51	14	Pyknic
11	32	8	40	0	Pyknic
12	50	5	55	16	Asthenic
13	46	14	60	16	Pyknic
14	44	11	55	0	Asthenic
15	43	15	58	12	Asthenic athletic
16	50	18	68	26	Pyknic
17	49	5	54	12	Pyknic
18	44	2	46	12	Asthenic
19	42	3	45	10	Asthenic
20	37	2	39	0	Asthenic
21	42	7	49	16	Pyknic
22	51	12	63	16	Pyknic
23	41	12	53	14	Pyknic
24	37	10	47	10	Asthenic
25	51	17	68	20	Pyknic
26	48	17	65	16	Pyknic
27	40	6	46	18	Pyknic
28	44	8	52	16	Pyknic

\* Cases 1 to 10

† Cases 11 to 28

had been between 31 and 40 years of age. As is known from the experience gained with other orally active hypoglycemic agents that have been studied failure of treatment is practically the rule in cases of diabetes of infantile, juvenile, and young adult types, this also seems to be the case with chlorpropamide. For example, if we take into consideration only those patients who had developed diabetes at 40 or more years of age (TABLE 4), we obtain

TABLE 7  
DATA ON 24 DIABETIC PATIENTS SHOWING FAIR\* AND POOR† RESPONSE TO CHLORPROPAMIDE

Case No	Age at onset of diabetes (years)	Duration of diabetes (years)	Age (years)	Insulin U/24 hours	Body type (constitution)
1	7	3	10	40	Asthenic
2	45	1	46	10	Asthenic
3	28	3	31	30	Asthenic
4	49	1	50	0	Asthenic
5	50	7	57	24	Asthenic
6	34	2	16	36	Pyknic
7	4	1	5	12	Pyknic
8	8	6	14	54	Asthenic
9	17	12	29	52	Asthenic
10	26	8	34	54	Asthenic
11	25	10	35	50	Asthenic
12	7	5	12	46	Asthenic
13	50	4	54	8	Pyknic
14	15	17	32	52	Asthenic
15	7	6	13	58	Asthenic
16	30	6	36	30	Asthenic
17	25	4	29	46	Athletic/asthenic
18	48	13	61	64	Pyknic
19	16	4	20	64	Athletic
20	16	9	25	48	Asthenic
21	27	6	33	30	Asthenic/athletic
22	15	8	23	60	Pyknic athletic
23	9	3	12	42	Asthenic
24	30	1	31	40	Asthenic

\* Cases 1 to 4

† Cases 5 to 24

83.3 per cent of satisfactory results; this percentage is only 13.5 in patients with diabetes developing before 40 years of age (TABLE 5).

Examination of TABLE 6, which shows data on patients satisfactorily controlled (excellent and good results) with chlorpropamide reveals that (1) nineteen (68.2 per cent) showed a body constitution of the pyknic type, (2) twenty five (89.2 per cent) developed diabetes at 40 or more years of age, and (3) except for 1 patient, none received more than 20 U of insulin every 24 hours.

On the other hand, TABLE 7, showing data on patients in whom unsatisfactory results were obtained, reveals that (1) only 4 patients (16.6 per cent) had a body constitution of the pyknic type, (2) in 19 cases (79.1 per cent), the diabetes had developed before 40 years of age, and (3) only 4 patients (16.6 per cent) needed less than 20 U of insulin per day for control.

#### *Tolerance and Side Effects*

Generally speaking, the drug was well tolerated at the doses used. One patient complained of epigastric distress, moderate pruritus without any apparent lesions was present in another. In a girl 10 years of age there was a slight decrease in the white blood cell count, but without concomitant neutropenia. In one instance, few red cells were found in the urinary sediment. A male patient 23 years of age, with severe diabetes, complained of somnolence, pruritus of the eyes, and moderate headache. However, we really do not know whether these symptoms were due to failure to control the diabetes during the observation period or to the use of the drug. Treatment of a young woman 31 years of age, to whom we gave chlorpropamide at a dosage of 500 mg 3 times a day, was discontinued after 5 days because of epigastric pain. Approximately 72 hours after discontinuance the patient had a rash all over the body, together with severe itching and slight fever (100° F). Antihistaminic drugs with small doses of prednisone improved the condition. However, 24 hours later, there was an exacerbation of the skin lesions. At this time a closer interrogation of the patient revealed that, as self-prescribed treatment for a slight sore throat, she had taken penicillin tablets during the fourth and fifth day of the chlorpropamide treatment. Allergist Marcel Granier was of the opinion that at least this second rash could be explained by sensitization to penicillin. With proper treatment the patient recovered in a few days.

#### *Summary*

A total of 52 nonselected patients were studied from 5 to 36 days. Excellent results were obtained in 10 cases (19.2 per cent), good results in 18 patients (34.6 per cent), and fair results in 4 cases (7.6 per cent). Results obtained were poor in 20 cases (38.4 per cent), however, it should be noted that of these 20 cases, 17 were patients under 40 years of age, 11 of whom had developed diabetes when they were less than 20, the other 6 had developed it in their 20s. As is well known, it is precisely in this type of diabetic that the oral hypoglycemic drugs have been less effective. It seems that, in a high percentage of diabetics who respond well to chlorpropamide, it is possible to maintain control with a dosage much smaller than that required for similar drugs. Many of the patients studied could be kept under control with only 125 mg administered after breakfast, lunch, and dinner—a total dosage of 375 mg. During treatment, 1 of the patients showed a few red blood cells in the urinary sediment, another complained of a slight pruritus without an apparent lesion, and Case 12 showed somnolence, ocular pruritus, and a slight headache. It is possible that these untoward effects were caused by a diabetic unbalance rather than by the direct action of the drug. One case

showed an intense dermatitis about 72 hours after having had to discontinue chlorpropamide because of epigastric distress nevertheless it should be noted that this patient ingested 3 tablets daily of oral penicillin during the fourth and fifth days of treatment with chlorpropamide

*Acknowledgments*

We thank Rafael Nicanor Silva and Lidia M. Fernandez for their help in the preparation of the manuscript and Ramon Cuevas for his technical assistance

## CONSIDERATIONS FOR CONTROL NORMS TO EVALUATE RESULTS OF TREATMENT IN DIABETES MELLITUS

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If a real picture of the diabetic problem is to be obtained it must be considered not only in terms of the present and in those of the individual but also because of the increasing incidence of this disease its longer duration and its hereditary characteristics in terms of the future and of mankind.

Before 1921 the average life expectancy of a diabetic was only 4-5 years after the disease had been discovered. Today thanks to the discovery of the hormone insulin by F. G. Banting and C. H. Best and to many advances in different fields of medicine it is possible to say that if a diabetic is well controlled his life expectancy will generally be the same as that of a non-diabetic of the same age and sex and that his illness should not be an obstacle to enjoyment of a useful life. However the question emerges: When can we say that a diabetic is well controlled? We do not intend to discuss this point at length; however it is frequently said that if a diabetic has no sugar in the urine or shows only traces of glycosuria and if his blood sugar remains near normal ranges he can be considered well controlled. This could be true in many cases but we frequently see patients whose diabetes could be considered well controlled according to these standards yet who suffer from different degenerative conditions that we think are of diabetic origin.

In other words we think that hyperglycemia or glycosuria or both are indexes of the diabetes but cannot be taken as measures of the severity of the disease. In fact we often see patients with diabetes of long duration who have had no proper medical care for years who show high values of sugar in the blood and in the urine and still are free from the so called diabetic complications. At the same time unfortunately we see patients who adhere faithfully to the doctor's instructions show no glycosuria have moderate blood sugar levels most of the time but in whom degenerative conditions of the kidney and retinal vessels can be detected. What is more important in many of these cases is that the diabetes or at least the hyperglycemia has not been present for a long time. We say this because we feel that diabetes represents a very complex metabolic disturbance of which glycosuria or hyperglycemia or both are manifestations—the most frequent perhaps but not always the most important.

With the discovery of orally active hypoglycemic agents it seems to us the problem has become even more complex.

One thing we already know in many diabetics blood sugar levels can be maintained between satisfactory ranges with the use of the above mentioned drugs.

However another question arises: Can these patients be considered well controlled simply because their blood sugar is normal or almost so? It is



our belief that we must wait five to ten years before we can describe the condition of these patients accurately

The situation that confronts physicians and patients in 1958 is quite different from that when insulin was first made available. Insulin is a biological product and at that time it was unique as a diabetic specific. In other words from now on the results obtained with any drug in cases of diabetes mellitus should be analyzed from the following points of view. Does it satisfactorily lower the blood sugar? Does it have a better value than insulin in the prevention of the degenerative conditions? Does it have any undesirable effects? Can it be given safely for years to diabetics who often have fragile kidneys and liver?

Briefly we feel that in order to obtain better data to judge the results obtained in the treatment of diabetes it will be worthwhile to determine the type of control (such as laboratory tests, X-ray films and electrocardiograms) that should be used and the frequency with which it should be employed. This would enable us to detect diabetic complications as early as these medical techniques are capable of uncovering them. It would be the only way to determine when to readjust our therapeutic approach without the need of waiting for the appearance of full fledged clinical pictures such as those we see in cases of obstructive arteriopathy, intercapillary glomerulosclerosis and advanced neuropathy. It is our feeling that we may not have been quite correct in our treatment of some of our patients simply because our main criterion has been that of attaining satisfactory blood sugar levels.

The problem is so complex and of such great significance that possibly it would justify the establishment of an international committee to coordinate the work of researchers in this field in different countries.

#### Summary

In view of the current nature of the problem of diabetes and its treatment the evolution of this condition and the complications attendant on it (particularly those of a degenerative type), I consider it important to establish general norms for such aspects as medical controls and collateral studies. These would permit not only judgment of the effectiveness of the treatment with reference to hyperglycemia or glycosuria or both but would also provide an opportunity to gather data on such factors as incidence, degree of seriousness and evolution of the so-called complications of diabetes in relation to the various types of treatment. At the same time in order to evaluate the action of new drugs administrable *per os* and capable of lowering the glucose levels (but not necessarily controlling other diabetic manifestations) it will be necessary to plan special studies to determine their control of hyperglycemia or glycosuria and also comparative studies of patients treated for years with the new drugs and of those patients treated with insulin. It will also be necessary to establish the type and frequency of tests for the purpose of eliminating collateral or untoward reactions (or those of any other nature) that the hyperglycemic drugs active by the oral route are capable of producing.

# THE CLINICAL RESULTS OF THE TREATMENT OF DIABETES MELLITUS WITH CHLORPROPAMIDF

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During the last few years numerous studies have been made to find an

However it is evident that the relatively low toxicity of the compounds especially of tolbutamide as well as their usefulness for a specific group of diabetics can be taken for granted. In a recent report<sup>1</sup> we summarized the results obtained with carbutamide and tolbutamide in the treatment of a group of 26 diabetics over a period of 21 months in general the results confirmed those of other investigators

Only 29 of the 35 patients have been treated for 4 months or more we therefore base our conclusions on these cases only. Although we think that the treatment of the remaining patients can be considered a success data on them will not be included in this report as proof or justification of the conclusions

Of the 29 diabetics treated 14 are female and 15 male 35 to 68 years old with an average age of 55.1 years

6 months before preparation of this paper) and 2 had diabetes of 18 years duration

In the males 35 to 68 years old with an average age of 52.1 years the average duration of the disease was equally advanced (6.1 years) with one case of 23 years and 4 of less than 6 months (FIGURE 1)

Of the 29 diabetics 14 had been treated with tolbutamide for 12 months or longer at doses between 500 and 2000 mg daily 10 used between 10 and 80 U of insulin daily (average 43 U) and only the 5 remaining cases were maintained on diet alone

In all cases the treatment with the new drug began abruptly after preliminary clinical examination and analytical tests Except for Case 9 on

two occasions, the treatment was ambulatory and without change in the daily routine or in the diet previously followed

Among the patients previously treated with insulin were two (cases 4 and 6) who were positively refractory to tolbutamide as we found in an earlier trial

Regarding the others, one female patient with a serious diabetic retinopathy began the treatment in a state of acidosis another had an acute anthrax infection another had a carcinoma of the upper lip that was operated after the first week of treatment and two had tuberculosis of the lung (one cavitory)

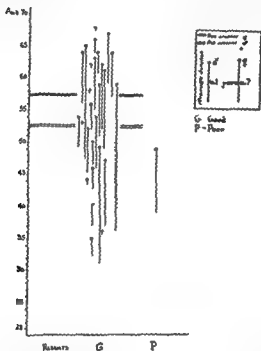


FIGURE 1 . Relation of age of patient and duration of diabetes to response to treatment.

One case of cortico-adrenal hyperplasia with metabolic hyperfunction of Cushing's type and another with a pituitary chromophobe adenoma should be mentioned. One patient developed dry gangrene of one foot during the sixth month of treatment but, in spite of this severe complication, we did not stop the medication. Thus far he has had good results with regard to blood glucose levels.

As coexistent illnesses, which may be considered minor as far as their relation to diabetes is concerned, essential hypertension was present in four patients and myocardial fibrosis in one.

With the exception of two patients, all belonged to the pyknic or pyknic athletic groups.

In addition to a complete clinical examination, the following analytical tests were made before the beginning of the trial

**Glucose tolerance curve** Using an oral dose of 25 gm glucose, analyses of blood glucose levels were made at 0-, 30-, 60-, and 120 min intervals. The curve was repeated 48 hours later with an oral dose of 1.75 gm glucose per kilogram of body weight. The blood glucose determinations were made by the Folin Kingsley Reynhold method until July and since then, by the Kingsley Reynhold Fister method.

**VPA determination** This was determined by the hypobromite method.

**Hanger's Takata tests and MacLagan's tests** In the majority of the patients and since June these tests have been performed by paper electrophoretic partition of serum proteins.

**Blood tests** Hemoglobin determination, hematocrit, red and white cell counts, and platelet count were carried out.

After beginning treatment repeated blood glucose determinations were made in order to maintain close watch over the effect of the medication. The other examinations were also repeated more or less frequently (an average of twice per month) in order to detect any possible systemic repercussions which might have developed. On the basis of the described technique we think that the conclusions reached can be accepted with reasonable confidence.

In the aforementioned experiments we endeavored to use the smallest initial dose possible. Although we administered the drug in divided doses until the middle of February, since then we have administered one dose daily at breakfast time.

The maximum daily dose was 2 gm (4 tablets), and was used by only 3 patients; the minimum dose in the initial treatment of 4 patients was 250 mg. In 9 cases we gave 500 mg; the average initial dose was 922 mg. With high doses we observed no toxic reactions except dizziness and vertigo in 4 cases; these reactions were coincident with hypoglycemia.

In general the doses—particularly those of 1 gm or more—were administered for short periods, usually not longer than 3 days. The single exception was Case 2, a patient with acidosis to whom we gave this dosage for 3 months.

The maintenance dosage usually reached at the end of the first week did not exceed 500 mg (4 cases). 12 patients were perfectly controlled on 125 mg daily, and for 4 a daily dose of 62 mg was sufficient. The average maintenance dosage was 248 mg.

In regard to food all patients followed a liberal diet of carbohydrates with quantities ranging between 300 and 400 gm per day. The other elements were relatively balanced. We took special care to recommend to the patients that they should not vary the qualitative or quantitative substance of their diet from what they had been following before the beginning of these studies.

In view of this we think that we can present the following conclusions with reasonable assurance. However, because of the short time and the limited number of patients, the results should be considered as preliminary.

#### Conclusions

- (1) Chlorpropamide proved active in patients who previously benefited from other types of sulfonylureas (carbutamide and tolbutamide).

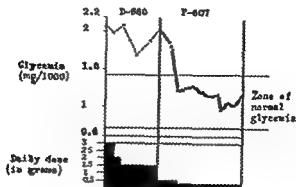


FIGURE 2

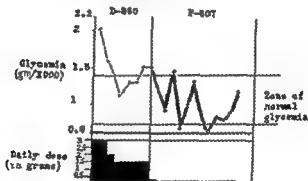


FIGURE 3

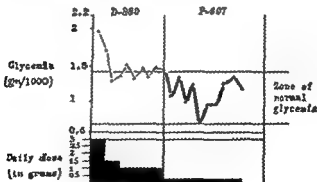


FIGURE 4

(2) Chlorpropamide was effective in two cases which had previously proved refractory to carbutamide and tolbutamide

(3) The clinical and metabolic regulation of diabetes with chlorpropamide is more simple and more complete than with other drugs tested so far (FIGURES 2, 3, and 4). In the present group of patients, some noteworthy differ

ences have been verified regarding the interrelation of the results obtained and the ages of the patients and especially the time of evolution of the disease. 37 per cent of the patients with good or excellent results are between the age of 61 and 68 (11 cases) with an average of 64. Greater differences occur in the relation with the duration of diabetes with 41 per cent of the patients having had the disease for 8 years or longer giving an average duration of 11.7 years (FIGURES 1 and 5). In our previous work with tolbutamide there were no good or fair results in the patients with diabetes of more than 8 years duration.

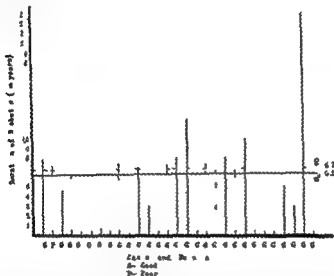


FIGURE 5. Duration of diabetes and response to treatment.

(4) In a study on 10 cases where the drug showed no effect and that were not included in the present report it was found that chlorpropamide did not control the disease of a patient 49 years old whose disease had begun at 39 and whose treatment started when he was in a state of marked diabetes.

This patient the only thin diabetic of the group had little diabetes requiring a daily minimum of 60 U of insulin. In spite of the fact that the patient had severe diabetic retinopathy, cataracts, and maintained the treatment for 31 months with doses varying between 1500 and 500 mg, rapid disappearance of ketonuria and the establishment of blood glucose at lower (although still abnormally high) levels than at the beginning were effected. From a systematic point of view there were no alterations worth recording except perhaps an improvement in visual acuity, normalization of the serum protein, and small changes in the leukocyte count.

5) The coexistence of a severe infection (anthrax or of latent or active tuberculosis) (in the latter case bilateral and cavitary) does not adversely affect the efficient and rapid therapeutic action of chlorpropamide although

it should be noted that the doses used were slightly higher than those used for the other patients in general. In one patient who developed hypoglycemia of one foot, the drug nevertheless controlled the hypoglycemia.

(6) On the average, the hypoglycemia was controlled by the drug.

In the case of the patient who developed hypoglycemia of one foot, the drug nevertheless controlled the hypoglycemia.

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subjective tolerance we must report that the hypoglycemia was controlled by the drug, and that the hypoglycemia was controlled by the drug, and that the hypoglycemia was controlled by the drug.

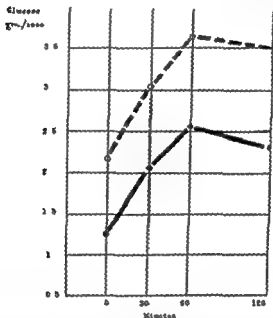


FIGURE 6. Glucose tolerance curve. Broken line represents tolerance before therapy with chlorpropamide; solid line after three months' therapy with chlorpropamide. Curves are averages of three cases previously treated with diet alone.

In one case a diabetic doctor had a severe melancholic depression, with suicidal ideas that promptly disappeared after the carbohydrate content of the diet was increased. No other neurological, digestive or dermatological changes were observed in any of the patients of this group. In one case not included in the present study, the administration of chlorpropamide caused the appearance of a nettle rash in a patient who previously had shown sensitivity to tolbutamide and the sulfa drugs in general.

Seven patients reported a sudden cephalic congestion after meals, with extrasystoles in two cases and mild lung edema in one—symptoms that disappeared promptly after stopping the intake of alcohol. One of those who later resumed drinking wine did not have the same symptom. We think the phenomenon may be due to a selective inhibition of acetaldehyde dehydrogenase.

(8) Analytical observations showed that the blood glucose level remained generally normalized within the first week of treatment. In some cases, where the patient spontaneously or upon our order did not adhere to the

the previous blood glucose level

An average of two urea determinations per month was made, and it was found that chlorpropamide did not increase the amount of nonprotein nitro

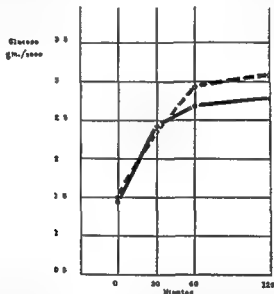


FIGURE 7. Glucose tolerance curve. Broken line represents tolerance before therapy with chlorpropamide; solid line after three months' therapy with chlorpropamide. Curves are averages of four cases previously treated with insulin.

gen (NPN). On the contrary, the NPN normalized itself or remained unchanged if previously high. It was found too that a value as high as 76 does not per se mean a contraindication.

Takata, Irias, Hangers, and MacLagan's tests did not show undesirable

to be responsible for this abnormal finding.

Serum protein electrophoresis was performed in twenty-one cases with normal results in all but one (Case 3).

No harmful repercussions were observed in the blood picture, which was generally tested with the same frequency as blood urea. On the contrary, we saw the normalization of the blood picture in the cases that previously had



shown alterations (6 cases) It is worth mentioning that 1 patient, who started treatment with a severe leukopenia, showed a progressive increase of the white cells from 2300 to 4900 during the trial The hematocrit value decreased in 6 patients

If glycosuria was present this disappeared with the normalization of the blood sugar The same applies to albumin in two cases and to ketone bodies in two others In one patient traces of albumin persisted No pathological

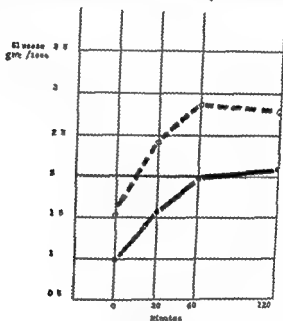


FIGURE 8 Glucose tolerance curve Broken line represents tolerance before therapy with chlorpropamide, solid line after three months therapy with chlorpropamide Curves are averages of seven cases previously treated with tolbutamide

changes in the sediment, especially crystalluria, and no alterations in the pH appeared

Glucose tolerance was investigated by means of an oral dose of glucose (1.75 gm/kg) and it was found that the 2 curves, obtained at intervals of 30 days or longer, can be considered (in their conjuncture) parallel (FIGURES 6, 7, 8, and 9) This shows that there is no modification in carbohydrate tolerance

It is our opinion that this new drug represents a major step in the treatment of diabetes mellitus<sup>2</sup> We know that more time, more patients, and above all more experience—clinical as well as analytical—are necessary in order to obtain conclusive knowledge about this therapy However, keeping in mind these limitations in comparison with the other drugs with which we have clinical experience (tolbutamide and carbutamide), we can say that chlorpropamide is more active and less toxic in the therapeutic dosage we have used

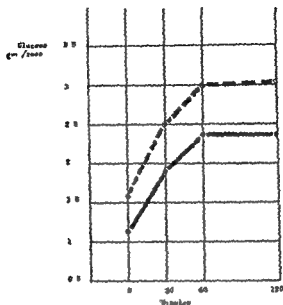


FIGURE 9 Glucose tolerance curve. Broken line represents tolerance before therapy with chlorpropamide; solid line after three months' therapy with chlorpropamide. Curves are averages of fourteen cases.

It is worth mentioning that all the patients were maintained on almost a free diet. During this clinical trial we did not observe significant variations in the maintenance dosage.

#### Acknowledgment

The authors wish to thank Ernesto Morais, who permitted his laboratory to be used for the analyses mentioned in this report. We also thank Pfizer International, Inc., New York, N. Y., M. Usano of that firm, and Neo Farmacéutica Lda, Lisbon, Portugal, for having placed at our disposal the quantities of chlorpropamide necessary for accomplishment of the study.

#### References

1. DE SALCEDO J & F BORGES. 1958. Algumas considerações sobre o tratamento por oral da diabetes mellitus pelos derivados sulfonamídicos. *J Méd* 25(787): 461-466.
2. DE SALCEDO J & F BORGES. 1958. Resultados preliminares do tratamento da diabetes mellitus pelo P 667 ou Chlorpropamide (Nota prévia). *Portugal med* 42 (1): 177-190.

## TOLERANCE FOR ALCOHOL IN PATIENTS ON CHLORPROPAMIDE

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By July 1, 1958 we had evaluated chlorpropamide clinically in 66 patients and had studied various sulfonylurea derivatives in approximately 600 patients with diabetes mellitus. By August 30, we had increased the number to 624 patients who had been treated for more than 1 month, of these, 88 had received chlorpropamide. Evaluating our results in this larger patient group, we can confirm the following five points relating to the control of diabetes mellitus with chlorpropamide.

(1) The hypoglycemic activity of chlorpropamide, particularly with maximum therapeutic dosages, is in some instances superior to that achieved with carbutamide or tolbutamide.

(2) The therapeutically effective dosage of chlorpropamide ranges from a maximum of 2 gm, which we used in some patients as an initial dose, to a minimum daily maintenance dose of 0.125 gm per day, these dosages were established on the basis of satisfactory daily control of blood sugar levels and the disappearance of sugar from the urine.

(3) We observed a favorable response in 72 of 88 patients (80 per cent) and an inadequate response in 16 patients (20 per cent). It is important to note that 15 patients were less than 40 years old at the time of initiation of therapy, and that 12 of these were benefited by chlorpropamide therapy. Only three showed an inadequate response.

(4) With the initiation of therapy, and at intervals thereafter, patients were given various laboratory tests including liver function tests, blood counts, measurement of azotemia, cholesterolemia, serum proteins, and serum transaminase. From the results obtained by these laboratory tests, we observed no evidence that therapeutically effective dosages of chlorpropamide exhibited any type or degree of toxic effects.

(5) Two types of side effects necessitated the discontinuance of therapy in some patients, all of whom belonged to the group that had not shown an adequate response to chlorpropamide. The side effects were manifested as episodes of sensitivity and individual intolerance similar to those observed in response to other sulfonylurea derivatives and as episodes of specific intolerance affecting the gastrointestinal tract (nausea, sensation of abdominal fullness, constipation).

Initially, our attention was drawn to the unusual side effect associated with the ingestion of alcohol during chlorpropamide therapy by the fact that eight of twenty-three patients who were under treatment complained that after meals they experienced one or more of a number of disagreeable symptoms that consisted of sensations of warmth, flushing, nausea, giddiness and, in some, tachycardia. The patients had already come to the conclusion that

these aberrant symptoms were probably caused by the chlorpropamide, which had recently been prescribed for their diabetic condition, since the unusual sensations appeared about three or four days following initiation of therapy.

The fact that these symptoms did not appear in all patients who were receiving chlorpropamide led us to the conclusion that this side effect was not due to the therapeutic action of the drug and therefore could not be considered a side effect that could be expected to occur as a direct consequence of its administration. We reasoned, therefore, that it must be due to some set of circumstances peculiar to the patients who had developed the

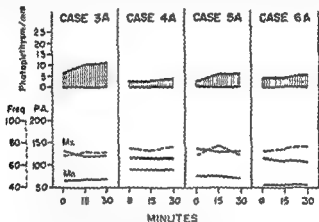


FIGURE 1. Alcohol test with normal subjects. Symbols P A, arterial blood pressure; Freq. PA, heart rate (broken line); Mx, maximum and Mn, minimum.

symptoms. We began to investigate other factors and discovered that the phenomena appeared exclusively in those patients who were accustomed to drinking wine regularly during their meals. We requested the patients to discontinue the ingestion of wine and their symptoms promptly disappeared. When they resumed wine with their meals, the symptoms returned.

While we have had other sulfonylureas under study for some time as our total patient group shows, we have never before observed such a striking reaction to the ingestion of alcohol during treatment with a sulfonylurea. It was necessary to discontinue chlorpropamide therapy in only one patient because of the development of this phenomenon. Of course had this patient been advised to discontinue the ingestion of alcohol during chlorpropamide therapy, his symptoms would have disappeared and we should have been able to continue him under chlorpropamide treatment. Such was not the case, however, and we substituted carbutamide for chlorpropamide three days after the initiation of chlorpropamide therapy. On carbutamide, this patient experienced alcohol intolerance symptoms similar to those he had experienced while on chlorpropamide but they were mild in nature and disappeared after a few days on continued therapy. Such a result corresponds to

the findings that some other investigators have reported concerning secondary effects observed in relation to the ingestion of alcohol by patients who were being treated with carbutamide or tolbutamide. Such symptoms of alcohol intolerance, however, have been infrequently observed with

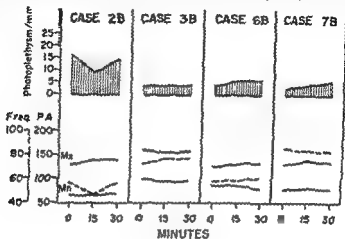


FIGURE 2 Alcohol test with diabetics not treated with chlorpropamide. Symbols as in FIGURE 1.

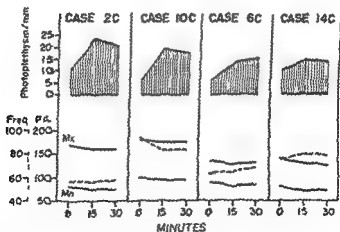


FIGURE 3 Alcohol test with diabetics under treatment with chlorpropamide. Symbols as in FIGURE 1.

these two drugs and are not considered to be of significance in their clinical application.

Following our observations of the eight patients who had experienced alcohol intolerance while under chlorpropamide therapy, we conducted the following clinical experiment:

Eight per cent (16 proof) ethyl alcohol was administered to all patients

in whom alcohol intolerance appeared. These patients had taken chlorpropamide for at least 8 days and therapy which had successfully stabilized their diabetic state was being continued. Within 10 to 20 min following the ingestion of the alcohol, these patients experienced an acute sensation of warmth of the face with flushing of the same area and in a few cases slight giddiness similar to that caused by ingestion of a large amount of alcohol.

None of these symptoms was observed when the same dose of alcohol was given to normal subjects or to diabetics being treated with other sulfonylurea derivatives.

This intolerance reaction to alcohol has been followed in all patients both before the ingestion by measurement of rate the auricular graphic pattern.

We have not observed any variation in the electrocardiographic pattern in any patient. The values for systolic blood pressure have shown a slight tendency to reduce usually about 10 mm Hg while diastolic pressure tended to reduce approximately 5 mm Hg. The pulse rates remained within normal limits and no significant variation from normal for each individual was observed. The more relevant data recorded related to the behavior of the auricular photoplethysmographic wave. FIGURES 1, 2, and 3 show the changes that occurred in each of the patients studied.

We can state that diabetics being treated with chlorpropamide show an intolerance to alcohol more frequently than the occasional occurrence that has been observed with other sulfonylureas. However, such side effects as do occur can be completely eliminated by discontinuing the ingestion of alcohol. Thus the patient may make a choice between ingestion of alcohol or continuing therapy with chlorpropamide.

## THE TREATMENT OF DIABETES MELLITUS IN AREAS OF NUTRITIONAL DEFICIENCY

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The hypoglycemic effect of sulfonamides and derivatives of sulfonylureas has been observed by Janbon *et al*<sup>1</sup> since 1942, but it was not until 1955 that systematic application of such findings to the treatment of diabetes mellitus was started (in France). In the same year, systematic clinical trials were undertaken in Germany by Fuchs.<sup>2</sup> Subsequently, the role of sulfonylurea derivatives in the management of diabetes mellitus has been extensively studied throughout the world.<sup>3-6</sup> While the mode of action remains to be further clarified, it is known that beneficial effects are obtained where the islets of Langerhans still possess some capacity to produce insulin.<sup>3,7</sup> Hence, these drugs find particular application in the relatively mild maturity-onset type of diabetes.<sup>8</sup>

Since most of the diabetics seen in the Philippines are of the maturity-onset type, the introduction of these oral hypoglycemic agents met with an enthusiastic response. This was somewhat dampened by the realization that carbutamide (BZ-55) had a relatively high incidence of side reactions, while the better tolerated tolbutamide (D-860) was a less effective hypoglycemic agent.

It is the purpose of this paper to describe our clinical studies on the new sulfonylurea derivative chlorpropanamide (P-607).

### *Clinical Material and Method*

The patients treated in the study described in this article were seen in the Diabetic Clinic of the Philippine General Hospital in Manila and in private practice. They represent part of the group used in the investigation with carbutamide or tolbutamide or both. A careful history was obtained and physical examination made in all instances. Prior to therapy, base line determinations included fasting blood sugar levels, urinalysis, renal function studies, hepatic function tests, and hematological and adrenocortical evaluation. Chest X-rays (for pulmonary tuberculosis) and cardiovascular studies were also done. The patients were seen in consultation by us at fortnightly intervals.

There was no uniform plan of therapy. Initial doses ranged from 500 to 2000 mg a day, while maintenance doses ranged down to 125 mg daily. The doses were adjusted according to the clinical and biochemical response of the patient.

The 51 patients in the series were observed for periods of from 6 to 20 weeks at the time of the preparation of this report.

*Clinical Results*

The response of a patient to therapy is difficult to gauge. The following arbitrary designations were adopted. The response was termed (1) excellent when the patient was rendered asymptomatic and where the fasting blood sugar level was lowered to and maintained within normal limits, (2) good

but where the fasting blood sugar, although not brought within 25 per cent

TABLE 1  
RESULTS OF THERAPY WITH CHLORPROPAMIDE

Response	No. of patients	Percentages
Excellent	14	27.46
Good	19	37.26
Fair	8	15.68
Poor	6	11.76
Discontinued treatment	4	7.84
Total	51	100.00

of the normal levels could be maintained at least at 60 per cent of the initial levels and (4) poor when no clinical or biochemical improvement could be demonstrated. In all instances side reactions were minimal. These criteria were adopted because in almost all instances some degree of lowering of the blood sugar was observed, although it could not be maintained.

TABLE 1 summarizes the results obtained with chlorpropamide in the 51 subjects studied. 64.72 per cent showed excellent to good results, 15.68 per cent had a fair response, 11.76 per cent were not benefited. Four patients, or 7.84 per cent, were forced to suspend medication for various reasons.

The following cases show the pattern of an excellent response. Case 2 (FIGURE 1), F. F., 69 years female was recognized as a diabetic only 1 month before treatment. She had the usual symptoms of polydipsia and polyphagia. Initial fasting blood sugar was 248 mg. per cent. Treatment was started with 1500 mg. of chlorpropamide divided into 3 doses. There was prompt disappearance of glycosuria. On the fourth day of treatment she complained of slight dizziness, headache and blurring of vision. Blood sugar was found to have dropped to 96 mg. per cent, and treatment was suspended. The disturbing symptoms promptly cleared. Returning slight glycosuria and rising fasting blood sugar to 130 mg. per cent indicated



toms subsided and blood sugar was maintained at 96 mg per cent. After about 1 month, chlorpropamide was suspended to check the need for further maintenance. There was a slight rise of FBS to 117 mg per cent in the next 3 weeks. Resumption of the medication resulted in lowering of the blood sugar without reappearance of the hypoglycemic manifestations.

Case 7 (FIGURE 2), A. M., 54 years, male, was known to be diabetic for  $2\frac{1}{2}$  years and had required 30 to 40 U of insulin for the past 2 years. An initial dose of 1000 mg chlorpropamide resulted in a precipitous drop of

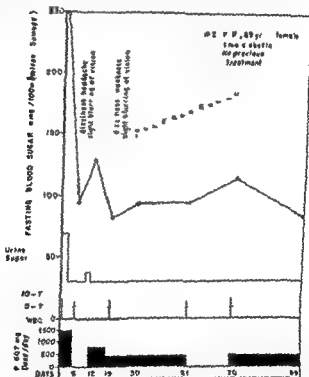


FIGURE 1

FBS from 210 mg per cent to 90 mg per cent in 2 days with accompanying dizziness, slight weakness, and unsteadiness of gait. These disappeared with suspension of treatment for about 5 days. Reinstitution of the drug at a dosage of 500 mg daily produced a slow decline in the FBS from 105 to 69 mg per cent in 31 days, without reappearance of side effects. Medication was suspended for 2 weeks and FBS rose to 85 mg per cent. Resumption of treatment at 250 mg daily and, later, 178 mg daily maintained blood sugar between 75 mg and 90 mg per cent.

FIGURE 3 shows an example of a good response. Case 19, D. T., 42 years, was found to have diabetes mellitus when he was confined for a pneumonitis. Initial blood sugar was 249 mg per cent. This was controlled with VPH

insulin at dosages reduced stepwise from 25 to a maintenance of 10 U daily. At the end of 1 month, FBS was maintained at 62 mg per cent, and insulin was suspended. After 1 week, FBS had risen to 125 mg per cent and carbutamide was given at levels of 500 mg daily. This caused a drop of the FBS to 75 mg per cent in the next 4 weeks. Carbutamide was suspended when FBS was reduced to 69 mg per cent. The patient was next seen about 2½ months later when he observed returning glycosuria. FBS had risen to 138 mg per cent, and carbutamide was resumed at 750 mg daily.

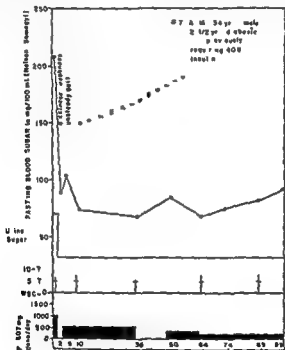


FIGURE 2

Blood sugar at previous visit to it at

glycosuria the FBS showed a slow tendency to rise to 77, then 94 and, finally, 112 mg per cent. The dose was then adjusted to 750 mg daily. Blood sugar showed a prompt fall. At one visit, a sudden elevation of FBS to 143 mg per cent was found to be associated with mental strain. Use of a tranquilizer without further adjustment of diet or increase in chlorpropamide dose, caused a return to the former state of control.

FIGURE 4 depicts the course of one of the youngest diabetics in our series. Case 20 O J, was first diagnosed at the age of 23 years. He is a male and

had pulmonary tuberculosis concomitantly. He did not receive any systematic form of treatment during the first 7 years of his diabetic life, and initial treatment consisted of 2000 mg carbutamide daily. In 2 weeks FBS dropped from 260 mg per cent to 143 mg per cent, and glycosuria declined from heavy to moderate. Carbutamide was reduced to 1500 mg daily because of the appearance of itching skin rashes. About 2½ months later, medication was suspended because of the reappearance of the cutaneous

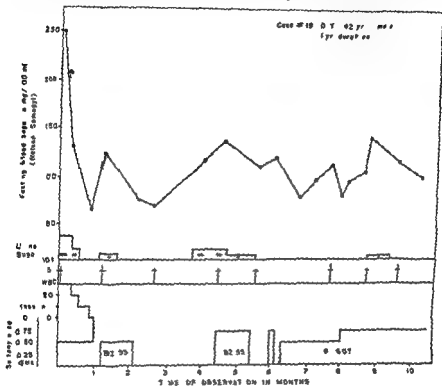


FIGURE 3

rash and a tendency to lowering of the leukocyte count. During this period, blood sugar remained about 130 mg per cent. After 1 month on antihistamines the skin condition had cleared and the white blood cell count (WBC) was normal; carbutamide was resumed. Failure to lower the FBS below 150 mg per cent, together with appearance of epigastric pain and diarrhea, caused suspension of carbutamide after 2 months. NPH insulin was given at levels of 15 U daily. This too, simply maintained FBS at the same level. After 2 more months the patient complained of the injections and requested another trial with oral hypoglycemics. Tolbutamide was then available and a trial was instituted at a dosage of 1500 mg daily. Gastrointestinal disturbance, sore throat and leukopenia ensued and caused immediate suspension of the drug; it was necessary to resume insulin. A

combination of 15 U NPH insulin and 1000 mg of carbutamide produced a progressive lowering of the blood sugar far more efficiently than could be produced by either drug alone

The insulin carbutamide combination was suspended when chlorpropamide became available. Started at 500 mg daily chlorpropamide proved insufficient and the dose was gradually increased to 1000 mg daily. Although the blood sugar could not be maintained at the same level as that obtainable with the insulin carbutamide combination the effects were definitely better

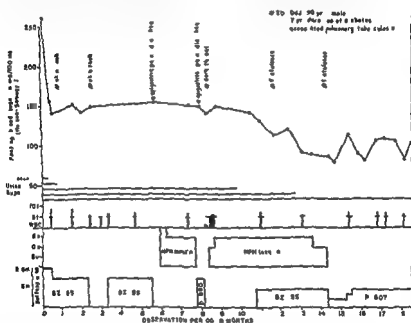


FIGURE 4

than with either 15 U of insulin or 1500 mg of carbutamide alone. We plan to combine a small amount of insulin with chlorpropamide.

FIGURE 5 shows an example of a poor response. Case 33 V I is a 51 year old male who had diabetes for 9 years and required 40 U of insulin for control. He showed evidence of hepatic dysfunction particularly in the abnormal results of flocculation tests. He was started on 750 mg of chlorpropamide daily. Because of lack of response dosage was increased to 1500 mg daily. There was a slight drop of FBS from 225 mg per cent to 148 mg per cent but without changing the dose or diet, the blood sugar could not be maintained at this level. The addition of 15 U of insulin arrested the upward spiraling of the blood sugar level but could not reduce it to normal levels.

FIGURE 6 represents the interesting case of a 58 year old female who was

known to be diabetic for 2 years, and had been treated with diet and insulin. She was admitted to the hospital for a lower abdominal mass, diagnosed by the Department of Gynecology as a cystadenoma of the ovary. Surgical intervention was projected, and the patient was referred for control of the diabetic picture prior to surgery. When first seen, she had a blood sugar level of 194 mg per cent, heavy glycosuria, and pruritus vulvae. She was placed on 2000 mg of chlorpropamide daily. FBS dropped to 112 mg per

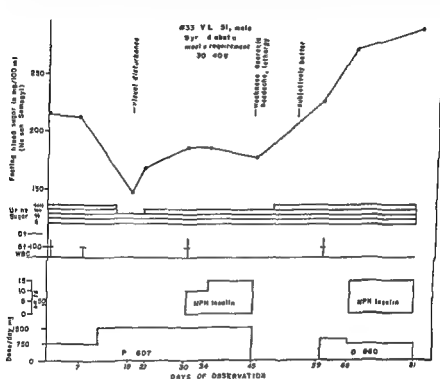


FIGURE 5

cent in 3 days and glycosuria was reduced to a minimum. A panhysterectomy was performed the next day, and the postoperative course was uneventful. The diabetic picture remained under control. Two weeks later it was necessary to reduce the medication to 1000 mg daily because of a transient weakness and dizziness. Attempts to reduce the dose further resulted in a rise of the blood sugar, a maintenance dose of between 750 mg and 1000 mg daily was found adequate.

The pattern of response of the patients treated in our study was very encouraging. There is a uniform tendency to lower the initial hyperglycemia and to maintain the lowered blood sugar level. FIGURE 7 depicts the relationship between the blood sugar level in patients whose response was rated fair to excellent and the amount of chlorpropamide required to

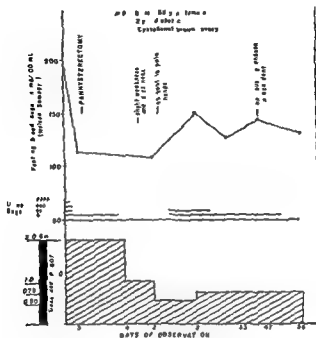


FIGURE 6

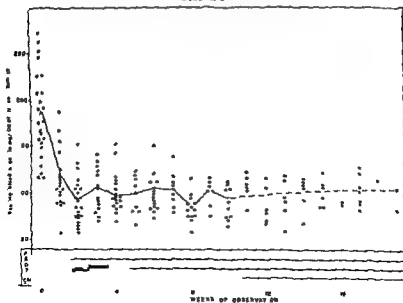


FIGURE 7

produce those results. Initial doses of slightly more than 1.5 gm daily were sufficient to produce a reduction of the fasting blood sugar from an average of about 180 mg per cent to about 118 mg per cent in 1 week. The fasting blood sugar could be maintained at about 100 mg per cent by maintenance doses as low as about 400 mg daily. These doses are certainly very much smaller than those we utilized previously with the other sulfonyl urea derivatives.

### *Side Effects*

The patients were carefully questioned at each visit about any untoward effects. TABLE 2 shows the side reactions that were encountered in the

TABLE 2  
SIDE REACTIONS OBSERVED WITH CHLORPROPAMIDE THERAPY\*

Reactions	No. of cases
Transient weakness	13†
Transient dizziness and headache	9
Transient blurring of vision	8
Unsteadiness of gait	3
Sialorrhea	3
Increased hunger pangs	2
Flatulence	2
Localized dermatitis	1
Exfoliative dermatitis	1†
Generalized allergy	1†
Leukopenia	0
Purpura or anemia	0

\* Duration of treatment of 51 patients: 4 months.

† Suspension of chlorpropamide therapy caused disappearance of symptoms.

51 patients during the period of observation. The most frequent complaints were those of transient weakness, dizziness, headache, and blurring of vision. They occurred with greatest frequency at the start of the investigation, when we used doses of chlorpropamide approximating those of tolbutamide. The complaints were often registered about 3 to 5 days after onset of treatment, and promptly cleared when medication was suspended. The effects have been attributed to a rapid reduction of the blood sugar levels as shown by close observations and repeated blood sugar checks similar to those depicted in FIGURES 1 and 2. Reduction of the initial doses of chlorpropamide to an average of 750 mg a day, with slight adjustment of the dose according to the individual response of the patient, diminished the frequency of the complaints in subsequent patients.

Four patients did not continue treatment. In 2 patients the appearance

fourth developed exfoliative dermatitis during the course of treatment. This is graphically shown in FIGURE 8. Case 36 III V 41 years female was known to be diabetic for 10 years and had an insulin requirement that varied from 20 to 40 U daily. She also had pulmonary tuberculosis and a family history of allergic tendency although the actual allergens could not be pinpointed. Initial FBS was 190 mg per cent and she was started on 750 mg chlorpropamide daily. Weekly visits thereafter showed progressive

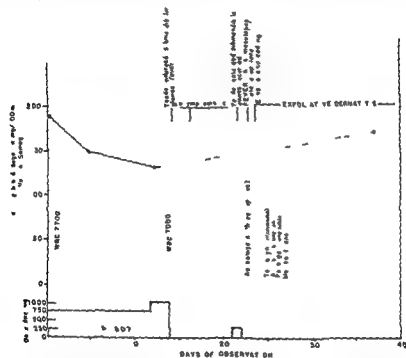


FIGURE 8

lowering of the blood sugar levels to 143 mg per cent and then to 130 mg per cent she was asymptomatic. On her second visit the dose was increased to 1000 mg daily. After 2 days she complained of slight fever and tender enlarged submandibular glands. Blood examination showed normal WBC. She was then advised to suspend chlorpropamide and all symptoms subsided 2 days later.

After one week the patient again felt tenderness and enlargement of the submandibular glands and slight fever. She took one tablet of chlorpropamide at noon and complained of heaviness of the body, higher fever, chills



continued to spread. Subsequent medication with Tetracyclin, Antistine and Peralga brought no relief and her condition suddenly became a full blown exfoliative dermatitis. When seen in the hospital, she appeared in critical stage and had to be treated with insulin and Melicortelone. The events do not with certainty indict chlorpropamide as the culprit, but the case is being presented as one where a dangerous skin disease occurred during the course of treatment. The patient recovered completely from the exfoliative dermatitis.

TABLE 3  
COMPARISON OF DIABETIC DEATH RATES OF DIFFERENT COUNTRIES

Country	Year	Population	Deaths from all causes	Deaths from diabetes	
				Number	Rate per 100 000
Australia	1949	7 912 638	75 260	1 473	18.6
United States	1950	150 697 000	1 450 270	25 010	16.6
Norway	1949	3 233 000	29 082	440	13.6
Canada	1950	13 821 000	123 649	1 545	11.2
France	1950	41 900 000	526 280	3 763	9.0
Italy	1950	46 279 500	448 971	3 544	7.7
Spain	1949	28 023 000	316 308	1 492	5.3
Mexico	1948	22 233 200	433 694	811	3.6
Colombia	1948	10 776 900	154 392	265	2.5
Japan	1950	83 200 000	908 891	2 007	2.4
Philippines	1956	21 856 875	219 719	137	0.6

TABLE 4  
INCIDENCE OF DIABETES MELLITUS AND DIABETIC ACIDOSIS IN PATIENTS AT THE  
PHILIPPINE GENERAL HOSPITAL MANILA PHILIPPINES, 1945-1955

Number of hospital admissions	181 116
Number of medical cases	32 112
Admissions and cases diagnosed as diabetes mellitus	619 (1.9 per cent)
Admissions and cases diagnosed as diabetic acidosis	10

TABLE 5  
COMPARISON OF INCIDENCE OF DIABETIC ACIDOSIS IN THE PHILIPPINES AND THE  
UNITED STATES

	Philippine General Hospital	New England Deaconess Hospital	Philadelphia General Hospital
Period covered	1945-1955	1946-1951	1931-1936
Number of diabetic admissions	639	10 200	3 009
Number of diabetic comas	10	153	268

TABLE 6  
COMPARISON OF DIETARY INTAKE AND DIABETIC MORTALITY RATES IN DIFFERENT COUNTRIES

Country	Total calories	Distribution of caloric intake								Diabetes mellitus mortality per 100 000
		Cereal	Root tubers	Sugar	Fats	Legumes	Fruits and vegetables	Meat	Milk	
Australia	3128	872	93	582	420	28	106	755	282	18.6
United States	3249	887	139	515	502	105	210	524	367	16.6
Norway	3129	1147	242	391	631	27	40	326	313	13.6
Canada	3109	943	177	518	464	81	118	442	366	11.2
France	3012	1203	247	247	520	100	67	303	222	9.0
Italy	2627	1606	63	80	256	158	52	148	108	7.7
Spain	2783	1416	180	143	360	158	86	324	101	5.3
Mexico	1909	1004	42	197	106	108	96	197	153	2.6
Colombia	1934	768	313	■	93	45	100	279	231	2.5
Japan	2268	1559	157	163	41	147	83	107	11	2.4
Philippines	2021	1115	264	124	140	79	23	218	68	0.6

There were no evidences of impairment of renal function or liver function, or of injury of the bone marrow in any of the cases under observation

### Discussion

Diabetes mellitus is not a major cause of death in the Philippines. TABLE 3 shows a comparison of pertinent figures available from different parts of the world.<sup>2</sup> Among the countries on this side of the Pacific Australia ranks high with diabetes mellitus accounting for 18.6 deaths per 100,000 population. Japan has only 2.4 per 100,000 population and the Philippines had only 1.37 deaths ascribed to the disease in 1956 or a mortality rate of 0.6 per 100,000 population.

Figures from the Philippine General Hospital (TABLE 4) for the period 1945 to 1955 show that 1.9 per cent of the patients admitted to the Department of Medicine were diagnosed as cases of diabetes mellitus; however of the 639 cases occurring during this period only 10 showed diabetic acidosis. In comparison (TABLE 5) 5-year surveys in the New England Deaconess Hospital, Boston, Mass. and the Philadelphia General Hospital, Philadelphia, Pa. show a large number of patients with diabetic acidosis.<sup>3</sup>

These statistics support the observation by most practitioners in the Philippines that the great majority of diabetics there are mild and that juvenile or growth-onset type of diabetes is a rarity. The factors that may explain this are not entirely clear. One suggestion that may be tenable is that the Filipino diet<sup>10</sup> which is relatively low in fat and moderate in calories approximates the diabetic diet. Inasmuch as dietary restriction is an accepted method of treatment it is postulated that the somewhat nutritionally deficient diet contributes to a milder course of the disease.

TABLE 6 shows a comparison of the general food habits from different countries as gleaned from Food and Agriculture Organization (FAO) reports.<sup>11</sup> There appears to be a rough correlation between the nutritional adequacy of the diet and the mortality rate for diabetes mellitus particularly with respect to the total calories and the fat content. The evidence while not conclusive, points to a trend which deserves further investigation.

The use of the sulfonylurea derivatives in diabetes mellitus ushers in a new era in management of the disease. This is especially true in areas where the diabetes found is mild and where resistance and the incidence of coma are low. Such conditions obtain in the Philippines and for this reason we have been very much interested in studying chlorpropamide.

### Summary

The great majority of cases of diabetes mellitus in the Philippines are of the maturity-onset type. The incidence of diabetic acidosis and of juvenile diabetes is small.

Chlorpropamide has shown great effectiveness in controlling diabetes mellitus.

The dose of chlorpropamide found effective in controlling the disease is much smaller than expected: initial dosages of 750 to 1000 mg daily and maintenance doses of 250 to 500 mg daily were found to be adequate.

Few side effects were encountered. The most frequent were symptoms of hypoglycemia produced when fairly large doses were employed.

A case of exfoliative dermatitis was reported to have developed in one patient during the course of treatment.

# References

- 1 JANSON M, J CHAPTAL, A VEDEL & J SCHAAP 1942 *Monipellher med* 21-22 441
- 2 FRANKF H & J FUCHS 1935 *Deut med Wochschr* 80 1449
- 3 MIRSKY I, A D DIENGOTT & H DOUGER 1956 *Science* 123 583
- 4 DUNCAN G G, C T LEE & J K LONG 1957 *Ann N Y Acad Sci* 71(1) 233
- 5 TRITS I B, J A MORTON, M WEINSTEIN & R LEVINE 1956 *Metabolism* 5 744
- 6 COLWELL A R & J A COLWELL 1956 *Metabolism* 5 749
- 7 LOUBATIERES A 1957 *Ann N Y Acad Sci* 71(1) 192
- 8 DUNCAN G G, C L JOINER & C T LEE 1956 *Metabolism* 5 946
- 9 JOSLIN F P, H F ROOT, P WHITE & A MARBLE 1952 *The Treatment of Diabetes Mellitus* 9th ed. 52, 342-343. Lea & Febiger, Philadelphia, Pa.
- 10 PASCUAL C, C L INTENDAN, J BULATAO, JAYME & E QUEGUE 1938 *The Statistical Repts* 2 6
- 11 WORLD FOOD SURVEY 1946 *FAO Rev*

# THE PROBLEM OF DIABETES IN THE SINGAPORE POPULATION AND THE IMPACT OF ORAL ANTIDIABETICS ON ITS MANAGEMENT

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In Singapore, where the population is made up of many races or ethnic groups and where literacy is far from universal, diabetes poses problems, particularly in management quite different from those met with in the more highly developed countries of America and Europe. Singapore has a population of 1,500,000, consisting mainly of Chinese (76.4 per cent), Malays, and Indians. It was from this population that subjects of this study were drawn and the investigation was carried out in 1 of the 2 teaching medical units in the Singapore General Hospital.

## *The Size of the Problem*

In 1957 the number of patients with diabetes newly registered in 1 of 2 diabetes clinics at the hospital totaled 377. These, together with patients already on the register, gave a total clinic attendance of 7,217 for the year. There is as yet no unified center for the treatment of diabetes, and such treatment is undertaken at the Singapore General Hospital, the Tuberculosis Hospital, outpatient clinics of the outpatient service, and by private practitioners. Therefore statistical data on the numerical size of the problem posed by diabetes mellitus are difficult to obtain. However, there is little doubt that diabetes constitutes a large part of the problem of the care of the health of the population.

## *Etiological Factors*

While definitive etiological factors in cases seen in Singapore and elsewhere may be largely similar, the emphasis on any particular etiological factor seems to vary. Thus, in Singapore, diabetes in children is decidedly uncommon, and the disease frequently manifests itself in middle life.

The maximum incidence falls within the age group of 36 to 65 years. The sex incidence also differs from that seen in other countries, as the disease is commoner among males than females. In its distribution among the various races, the disease is commoner among Indians in a population predominantly Chinese. The majority of patients comes from the low income group in which carbohydrates, especially in the form of rice, constitute the major part of the diet.

## *Presenting Symptoms*

Relatively few of our patients come to us because sugar was found in the urine on routine examination. Thirst and frequent passage of large quantities of urine, especially at night, comprise the symptoms most frequently complained of, while not a few come for medical advice because they observed

ants gathering about their urine or the toilet. Infective complications chiefly carbuncle and pulmonary tuberculosis cause them to be seen by our surgical colleagues or in the tuberculosis hospital. While a wide variety of complications is encountered the incidence of diabetic coma is definitely low. In one of the two medical units at the General Hospital only seven patients in diabetic coma were seen in the past year. This is not surprising as the great majority of the diabetics have diabetes of late onset.

#### *Problem in Management*

It is generally known that the Chinese culture emphasizes good food and in Singapore this attitude toward food is shared by the other communities. Hence it is extremely difficult if not impossible to expect our patients to adhere to a strict diabetic diet for long. It is chiefly for this reason that hospital inpatient treatment with its dietary regimen is unsatisfactory, as the patient's home food differs both in quality and quantity from that given him in the hospital. Consequently the disease is treated largely on an outpatient basis where the patient is allowed a free diet. Only those with infective and other severe complications are admitted to the hospital wards. In outpatient treatment while sugar and sweetened foods are strictly prohibited other carbohydrates (including rice) are offered at each meal. In this way our patients are able to arrive at a fairly constant carbohydrate intake frequently higher than the intake they can be allowed on dietary treatment alone.

As observed earlier the majority of our patients come from the poorer segment of the population and management of the disease and the initiation into a diabetic life are rendered more difficult by their illiteracy. Printed instructions are of little value. Each patient must be instructed in turn and where possible if language difficulties can be overcome some understanding of the disease process must be brought within his grasp. If this is achieved the illiterate diabetic will adhere faithfully to the advice of the physician and his disease will be brought under control quickly. Until the advent of oral drug therapy the overwhelming majority of patients received insulin treatment and were carefully taught to inject themselves and to test and chart their urinary sugar results.

As soon as it became generally known that tablets were available for the treatment of diabetes almost every patient saw in this a relief from daily injections. The demand for oral therapy was persistent and almost universal in the diabetes clinics and carbutamide and tolbutamide were tried and reported on by Tan Bock Yam and Ronald Wells (1957).

#### *Clinical Trial with Chlorpropamide*

In Singapore when chlorpropamide became available for investigational use it was decided to conduct a clinical trial in an attempt to assess its efficacy in the treatment of diabetes mellitus.

*Material* There were 100 patients who comprised a relation to the

However it was felt that patients with infective complications or acidosis or both should be excluded as the trial was conducted on an outpatient basis so that direct supervision could not be as close as when patients were hospitalized.

The age of patients at the onset of diabetic symptoms and their sex and race distribution are shown in TABLES 1 and 2.

TABLE 1  
AGE AT ONSET OF DIABETES AND SEX DISTRIBUTION OF PATIENTS ON CHLORPROPAMIDE

Age*	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70	Total
Male	2	6	—	7	6	8	4	1	1	42
Female	0	0	5	5	9	3	1	1	1	25
Total	2	6	12	12	15	11	5	2	2	67

\* Average age at onset of diabetes 45.8 years

TABLE 2  
RACE DISTRIBUTION OF PATIENTS ON CHLORPROPAMIDE

No. of patients*					
	Chinese	Indian	Malay	Others	Total
Group A	12 (44.4)	13 (48.1)	11	2 (5)	27 (100)
Group B	11 (44.0)	9 (36.0)	4 (16.0)	1 (4.0)	25 (100)
Group C	2 (13.3)	9 (60.0)	4 (26.7)	1	15 (100)
Total	25 (37.3)	31 (46.3)	19 (28.3)	4 (6.1)	67 (100)

\* Figures in parentheses are percentages of total

In the evaluation of the therapy, Group A were treated with tolbutamide and Group C those treated with insulin prior to chlorpropamide therapy.

All patients were examined clinically and urinary glucose tolerance tests X-ray examinations of the chest and blood counts were done the first and last were repeated at intervals.

The patients attended the clinic weekly while dosage of chlorpropamide was adjusted according to the urinary sugar reaction to Benedict's reagent the urinary tests (checked by the laboratory at intervals) were done by the

TABLE 3  
CLINICAL DATA OF 27 DIABETIC PATIENTS (GROUP 1) NOT ON ANY PREVIOUS TREATMENT

Case No.	Race	Sex	Present age (years)	Age at onset (years)	Blood sugar mg		Chlorpropamide gm/day	Duration of treatment (weeks)	Quality of control	Side effects	Thrombocytes per cu mm blood	Remarks
					Fa	Ma						
1	Ind	M	33	32½	133	266	0.5	22	good	Paron let g/dl neg	180 000	
2	Ind	M	50	50	246	410	0.75	14	good	g/dl neg	—	
3	Ind	M	44	41	282	342	0.7	23	good	g/dl neg	150 000	
4	Ch	P	53	53	233	302	1.0	17	fair	negative decem r	110 000	random blood sugar 130 mg %
5	Ind	M	55	53	306	540	0.5	15	good	n.t.	225 000	random blood sugar 61 mg %
6	Ch	M	56	49	216	294	0.5	14	good	shapiness n.t.	135 400	
7	Ch	P	54	54	218	310	0.75	1	good	const. let on g/dl neg	190 000	
8	Ind	M	54	49	216	312	0.5	0	good	const. let on g/dl neg	110 000	ata n.t.
9	Ind	M	54	54	263	400	0.5	4	good	n.t.	80 000	
10	Ch	P	51	50	204	314	0.8	15	good	n.t.	200 000	
11	Ch	P	49	49	86	217	0.23	10	good	anxiety n.t.	80 000	hypertension
12	Ch	M	60	64	210	—	0.75	10	good	n.t.	80 000	palpitation
13	Ind	M	32	32	90	256	0.5	2	good	fat	—	
14	Ind	M	33	33	184	240	0.5	8	good	fat	—	
15	Ch	P	40	40	172	208	0.5	6	good	fat	—	
16	Ind	P	46	46	252	370	1.0	4	poor	fat	—	
17	Ch	P	51	46	—	—	—	—	—	fat	—	
18	Ind	M	33	37	233	449	0.5	7	fat	fat	—	
19	Ind	M	39	30	234	370	0.75	5	good	const. let on	—	ferret
20	Ch	M	52	52	126	212	0.25	3	good	n.t.	—	
21	Ind	M	34	34	227	392	1.0	4	good	n.t.	—	
22	Ch	M	44	37½	237	392	0.5	3	good	const. let on	250 000	pat phthal neuritis
23	Ind	M	44	42	184	302	0.75	3	good	n.t.	137 000	
24	Ind	P	40	45	238	366	1.0	4	poor	n.t.	190 000	appendicitis
25	Ch	M	58	58	—	—	0.5	6	good	n.t.	60 000	atact both eyes
26	Ind	M	38	38	—	—	0.5	8	fat	n.t.	—	
27	Ch	P	40	39	—	—	0.5	8	fat	n.t.	—	

\* Key: Ind Indian, Ch Chinese, Eur European, P Filipino

† In glucose tolerance test



TABLE 4  
CLINICAL DATA OF 25 DIABETES PATIENTS (GROUP B) PREVIOUSLY TREATED WITH TOLBUTAMIDE

Case No.	Refer-ence	Race*	Sex	Pre-ent age	Age at onset	Blood sugar mg %		Total dosage		Duration of treatment		Quality of control		Side effects		Thrombocytes per cu mm blood	Remarks
						Fasting	Max†	Chlor-prop- amide gm/day	Tol-but- amide gm/day	Chlor-prop- amide (weeks)	Tol-but- amide (months)	Chlor-prop- amide	Tol-but- amide	Chlorprop- amide	Tol-but- amide		
28	1878/56	Ch	M	67	62	130	340	1.0	1.0	2	12	poor	good	weak tired anorexia	n l	—	patient refused to continue w. th chlorpropamide
29	N 812	Malay	M	51	28	220	370	0.75	0.5	11	18	fa r	fa r	n l	n l	130 000	
30	2355/46	Ind	M	52	44	283	444	0.5	1.5	12	16	fa r	fa	n l	n l	140 000	
31	N 1413	Ch	M	52	47	233	396	0.25	1.5	6	9	good	good	erythema toxic rash	n l	120 000	
32	N 5145	Ch	P	37	37	106	243	0.75	3.0	6	3	fa r	good	erythema toxic rash	n l	230 000	
33	462/37	Ind	M	54	53	256	555	0.75	1.5	4	2	poor	poor	purpura	n l	—	unreliable
34	N 533	Ch	P	52	50	—	—	1.0	1.5	9	8	good	good	erythema toxic rash	n l	180 000	
35	N 1772	Ind	M	46	42	142	285	0.5	1.0	12	3	good	good	erythema toxic rash	n l	60 000	
36	1594/37	Ch	P	55	48	—	—	0.75	1.5	5	12	fa r	poor	const. pat on	n l	—	
37	N 1127	Ind	M	64	60	200	—	0.5	1.5	12	6	good	good	const. pat on	n l	200 000	
38	2307/56	Malay	M	37	35	200	284	0.75	1.5	12	15	better	good	const. pat on	n l	210 000	
39	N 1354	Ch	M	57	53	200	245	0.75	1.5	11	8	good	good	const. pat on	n l	160 000	
40	451/57	Ch	P	47	46	—	—	0.5	1.5	13	11	good	good	const. pat on	n l	—	
41	N 1367	Ch	P	74	69	120	240	0.5	1.5	2	4	poor	good	const. pat on	n l	—	
42	N 908	Ch	P	52	52	152	270	0.75	1.5	9	16	good	good	const. pat on	n l	150 000	chlorpropamide d. acount used
43	N 655	Ind	M	56	54	550	714	0.5	0.5	10	11	good	good	const. pat on	n l	150 000	random blood sugar 123 mg % chlorpropamide d. acount used
44	N 597	Ch	P	56	44	250	362	1.0	3.3	6	7	fa r	good	const. pat on	n l	—	
45	604/56	Ind	M	54	51	178	302	1.0	2.5	9	12	good	good	const. pat on	n l	180 000	random blood sugar 123 mg % chlorpropamide d. acount used
46	N 6512	Ch	P	50	46	356	540	1.0	2.5	7	10	good	good	const. pat on	n l	110 000	
47	705/56	Ch	P	46	41	260	316	0.5	1.5	9	13	good	good	const. pat on	n l	160 000	
48	N 595	Malay	P	47	45	—	—	0.25	0.5	7	6	good	good	const. pat on	n l	130 000	random blood sugar 153 mg %
49	N 637	Malay	P	56	55	224	354	1.0	1.5	6	13	good	good	const. pat on	n l	—	
50	N 503	Ind	P	56	63	212	392	0.75	1.5	13	18	good	good	const. pat on	n l	200 000	
51	1090/46	Ch	P	53	49	167	350	0.75	1.5	6	10	good	good	const. pat on	n l	—	
52	N 613	Ind	M	55	51	—	—	0.75	1.5	5	10	good	good	const. pat on	n l	—	

\* Key: Ch Chinese; Ind Indian; Cey Ceylonese.  
† In glucose tolerance test

TABLE 5  
CLINICAL DATA OF 15 DIABETES PATIENTS (GROUP C) PREVIOUSLY TREATED WITH INSULIN

Case No.	Refer- ence No.	Race*	Sex	Present age	Age at onset	Blood sugar mg %		Prenal dosage		Duration of treatment		Quality of control		Chlor- propamide side effects	Insu- lin shock	Throm- bocytes per cu mm of blood	Remarks
						Fasting	Max	Chlor- prop- amide gm/day	Insulin U/day	Chlor- prop- amide (weeks)	Insulin (monthly)	Chlor- prop- amide	Insu- lin				
33	1701	Ind	M	43	42	160	362	1.5 P21.46	51.40 P21.46	5	11	poor	good	edema, weight loss, anorexia, lassitude, constipation	nil	—	chlorpropamide discontinued
34	1704	Ch	M	64	60	—	—	0.75 P21.24	51.40 P21.24	20	5 1/2	good	good	—	coma once	310 000	retinopathy and cataract
35	1712	Ind	M	33	33	280	362	0.25 P25.12	125(L)28 P25.12	5 1/2	2	good	poor	nil	—	165 000	random blood sugar 91 mg %
36	1786	Ind	M	51	50 1/2	206	388	1.0 P25.14	125(L)40 P25.14	9	5	good	poor	edema, anorexia	coma once	140 000	insulin gradually withdrawn
37	1664	Ind	M	45	44	174	312	1.0 P25.14	125(L)36 P25.14	30	9	good	fair	nil	—	160 000	random blood sugar 111 mg %
38	1775	Ch	M	39	39	146	—	1.0 P25.14	125(L)24 P25.14	33	2	good	fair	nil	nil	150 000	random blood sugar 111 mg %
39	2134/37	Malay	M	48	45 1/2	166	332	0.75 P21.28	125(L)44 P21.28	9	2 1/2	good	fair	nil	nil	140 000	random blood sugar 86 mg %
40	1724	Malay	M	39	39	241	326	1.0 P21.28	125(L)44 P21.28	9	—	good	fair	nil	nil	200 000	random blood sugar 156 mg %
41	2135/36	Ind	M	61	55	—	—	1.0 P21.28	51.42 P21.28	9	5 1/2	good	fair	anorexia	nil	—	random blood sugar 139 mg %
42	1989	Ind	M	45	42 1/2	184	302	0.5 P25.14	125(L)40 P25.14	11	18	good	good	initial edema	nil	140 000	random blood sugar 77 mg %
43	1013/37	Malay	M	34	43	180	290	0.5 P25.14	125(L)36 P25.14	11	11 1/2	good	fair	constipation	nil	195 000	random blood sugar 77 mg %
44	1497	Malay	P	41	48	303	454	0.25 P25.14	125(L)22 P25.14	9	26	good	good	nil	nil	165 000	—
45	1607/36	Ind	M	67	64	126	255	0.75 P21.24	125(L)32 P21.24	8	18	good	good	constipation	nil	150 000	—
46	1319/36	Ind	P	42	35	147	326	1.0 P21.24	125(L)27 P21.24	5	30	fair	poor	nil	nil	—	—
47	1319/36	Ind	P	50	57	203	352	1.0 P21.24	125(L)24 P21.24	5	5	fair	good	nil	nil	140 000	—

\* Key: Ind, Indian; Ch, Chinese.  
† In glucose tolerance test.

patients three daily—before breakfast and after lunch and dinner. Patients were given full clinical examination whenever they complained of any symptoms that might be construed as probable side effects of the drug, and they were questioned directly for any symptoms referable to the cardiovascular, digestive, urinary, and neurological systems and about any skin reaction.

Patients who were on insulin or tolbutamide, regardless of the quality of control achieved, had their insulin or tolbutamide discontinued for a variable

TABLE 3  
CORRELATION BETWEEN AGE AT ONSET OF DIABETES AND CONTROL ACHIEVED

Quality of control	No. of patients per age group*									Total
	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70	
Good	1 (50)	0 (85.7)	6 (60.0)	9 (75)	13 (85.6)	0 (69.2)	3 (75)	1 (50)	1 (50)	49 (73.1)
Fair	1 (50)	1 (14.3)	3 (30)	2 (16.6)	1 (7.2)	2 (15.4)	1 (25)	nil	nil	11 (16.4)
Poor	nil	nil	1 (10)	1 (8.4)	1 (7.2)	2 (15.4)	nil	1 (50)	1 (50)	7 (10.5)
Total	2 (100)	7 (100)	10 (100)	12 (100)	15 (100)	15 (100)	4 (100)	2 (100)	2 (100)	67

\* Figures in parentheses are percentages of total

period until glycosuria indicated a relapse of the diabetic state. Chlorpropamide was then given in place of insulin or tolbutamide.

**Dosage of chlorpropamide.** As the full toxicology of chlorpropamide in man is not known, it was decided to start treatment with the drug in a relatively small dosage given once or twice daily for the first week. Initially, 0.5 gm. of the drug was given, but this was reduced to 0.25 gm., the smaller dose being less likely to produce side effects. The dosage of 0.25 gm. was increased at weekly intervals to 2, 3 or even 4 times daily until control was achieved or until side effects necessitated the discontinuance of treatment. Once control was established, the dosage was reduced to the maintenance level, as adjudged by the relative absence of glycosuria.

**Classification of clinical data.** Taking into consideration the renal threshold for glucose in the individual patient, the quality of control of the diabetes was assessed on the basis of the degree of glycosuria present. A condition with no sugar in the urine, or only infrequent traces of sugar was designated good. Where traces of sugar in the urine occurred frequently, after dietary deviations had been excluded, the control of the diabetes was classified as fair. Greater glycosuria constituted poor control. The clinical data are presented in TABLES 3, 4, and 5.

TABLE 7  
CORRELATION BETWEEN SEX AND RACE AND CONTROL ACHIEVED

Quality of control	Sex		Race				Total
	Male	Female	Chinese	Indian	Malay	Others	
Good	14 (50.9)	15 (60)	16 (64)	23 (74.2)	7 (87.5)	3 (100)	49 (73.1)
Fair	4 (9.5)	7 (28)	1 (16)	6 (19.4)	1 (12.5)	nil	11 (16.4)
Poor	4 (9.6)	3 (12)	5 (20)	2 (6.4)	nil	nil	7 (10.5)
Total	42 (100)	25 (100)	25 (100)	31 (100)	8 (100)	3 (100)	

\* Figures in parentheses are percentages of total

TABLE 8  
CORRELATION BETWEEN DOSAGE OF CHLORPROPAMIDE AND CONTROL ACHIEVED

Quality of control	No. of patients per chlorpropamide dosage gm *					Total
	0.25	0.5	0.75	1.0	1.5	
Good	7 (10.4)	17 (25.4)	14 (20.9)	11 (16.4)	nil	49 (73.1)
Fair	nil	1 (1.4)	4 (6)	4 (6)	nil	11 (16.4)
Poor	nil	1 (1.5)	2 (3.0)	3 (4.5)	1 (1.5)	7 (10.5)
Total	7 (10.4)	21 (31.3)	20 (29.9)	18 (26.9)	1 (1.5)	67 (100)

\* Figures in parentheses are percentages of total

*Analysis of clinical data* Analysis of the clinical data reveals that of the 67 patients treated with chlorpropamide, 49 (73.1 per cent) showed the diabetes (glycosuria) under good control. In 11 patients (16.4 per cent) the control was fair, and in 7 (10.5 per cent) the control was poor.

Correlation between the quality of control achieved under treatment and any one of the following factors—age at onset of diabetes, race, sex and dosage of chlorpropamide used—is given in TABLES 6, 7, and 8.

## Discussion

*Hypoglycemic activity of chlorpropamide* There is no doubt that chlorpropamide is an effective hypoglycemic agent, as it was able to reduce glycosuria to a minimum in 73.1 per cent of the cases studied. This segment is made up of the 49 good control patients, whose ages at the onset of diabetic symptoms ranged from 30 to 66 years. The degree of control of the diabetes, when related to the ages of the patients at the onset of diabetic symptoms (TABLE 6) does not reveal with any certainty that the drug is more effective in the older than the younger age group, 85.7 per cent of patients between the ages of 31 and 35 achieved good control of glycosuria, while 75 per cent between the ages of 56 and 60 also had good control.

TABLE 9  
CORRELATION BETWEEN FASTING BLOOD SUGAR AND CONTROL ACHIEVED

Quality of control	No. of patients per fasting blood sugar levels mg per cent*		
	50-150	151-250	251-350
Good	5 (55.6)	29 (66.3)	7 (77.7)
Fair	2 (22.2)	5 (13.1)	2 (22.3)
Poor	2 (22.2)	1 (10.6)	nil
Total	9 (100)	35 (100)	9 (100)

\* Figures in parentheses are percentages of total

The data of TABLE 7 where the sex of the patients is related to the degree of control of the diabetes shows that 80.9 per cent of male and 60 per cent of female patients achieved good control. It would seem that chlorpropamide is somewhat more effective in male than in female diabetics, but a definite pronouncement on this problem cannot be made without further investigation in a larger series of cases.

In relating the ethnic groups of patients to the number of cases with good control of glycosuria it is obvious that chlorpropamide is equally effective in Chinese, Indian, and Malay.

*Chlorpropamide dosage* The dosage of chlorpropamide required to maintain good control ranged from 0.25 gm. to 1.0 gm. daily, with an average total daily requirement of 0.647 gm.

The majority (31 patients) of good-control cases required a maintenance dose of 0.5 to 0.75 gm. daily, these cases constituting 46 per cent of the entire series and 63 per cent of all good-control cases. With the exception

of 1 patient in the poor control group, to whom a daily total dose of 1.5 gm of chlorpropamide was given. It is noteworthy that the total daily drug dosage in the fair-control and poor control groups ranged from 0.5 to 1.0 gm.

It may be that further increase in the drug dosage for these two groups will reduce further the glycosuria of a few patients.

Correlation studies between the fasting blood sugar level and the quality of control achieved under treatment do not indicate that patients with a high

TABLE 10  
CORRELATION OF FASTING BLOOD SUGAR TO CHLORPROPAMIDE  
DOSAGE IN GOOD-CONTROL CASES

Chlorpropamide dosage gm	No. of patients per fasting blood sugar levels mg per cent*			Total
	50-150	151-250	251-350	
0.25	1 (14.2)	3 (11.1)	2 (28.4)	6
0.5	4 (57.4)	9 (33.3)	3 (43.2)	16
0.75	1 (14.2)	9 (31.3)	1 (14.2)	11
1.0	1 (14.2)	6 (22.3)	1 (14.2)	8
Total	7 (100)	7 (100)	7 (100)	41

\* Figures in parentheses are percentages of total.

fasting blood sugar level necessarily required higher dosage to attain good control of their glycosuria. Neither does a low fasting blood sugar level indicate a low-dosage requirement to obtain good control. Thus the fasting blood sugar range of good control cases is 86 to 350 mg per cent, and 27 of these 41 patients (65.8 per cent) required a maintenance dosage of 0.5 to 0.75 gm daily. Correlation between the fasting blood sugar level and the quality of control achieved under treatment is shown in TABLE 9; the relationship between the fasting blood sugar level and the dosage of chlorpropamide required to achieve good control is shown in TABLE 10.

*Patients previously on tolbutamide therapy.* In Group B (TABLE 4) of this trial series 25 patients were treated with chlorpropamide after glycosuria returned or became more severe when tolbutamide therapy was discontinued. In this group 9 patients (36 per cent of the group) were subsequently better controlled on chlorpropamide than on tolbutamide, and 4 (16 per cent) were not as well controlled as with tolbutamide. The remaining 12 patients (48 per cent) had as good control with chlorpropamide as with tolbutamide.

Included in the 4 cases (Nos 28, 32, 41, and 44) in which chlorpropamide was less effective than tolbutamide was 1 patient (No 28) who was given chlorpropamide for 2 weeks, at which time he refused to continue treatment with the drug because of side effects. Control was subsequently re established with tolbutamide.

A comparison of the dosage of chlorpropamide with that of tolbutamide in patients whose glycosuria was controlled to a similar degree shows the average dose to be 0.66 gm for chlorpropamide and 2.0 gm for tolbutamide. Thus weight for weight chlorpropamide is a more effective hypoglycemic agent than tolbutamide.

*Patients previously on insulin therapy.* Patients on insulin therapy before chlorpropamide treatment constitute Group C (TABLE 5) of this series. Of the 15 patients of this group, 9 (60 per cent) achieved substantially better control of their diabetes than on insulin treatment. The insulin dosage ranged from 28 to 136 U daily, with an average of 63.5 U; the average dose of chlorpropamide was 0.83 gm. In 4 patients (26.6 per cent) chlorpropamide did not reduce the glycosuria any more than did the insulin given previously. In the remaining 2 patients (Nos 53 and 67) treatment with chlorpropamide resulted in poorer control than with insulin. In 1, the chlorpropamide dosage was increased to 1.5 gm daily while, in the other, the dose was increased to 1.0 gm daily. In both cases, the chlorpropamide was subsequently withdrawn and insulin resumed; good control was re established.

*Side effects of chlorpropamide.* Throughout the study with chlorpropamide, a close watch was kept for any side effects that might be considered as due to the drug action. Of the 67 trial cases, 39 (58.2 per cent) had no side effects of any description, even on direct inquiry. Five patients (7.5 per cent) reacted strongly to the drug (Nos 17, 28, 41, 44, and 53), and severe giddiness, fatigue, anorexia, constipation, nausea, and vomiting necessitated discontinuance of chlorpropamide. The remaining 23 patients (34.3 per cent) had mild side effects, most of which disappeared spontaneously even when the drug was continued, frequently with increased total daily dosage. The distribution of the mild symptoms complained of by the 23 patients (some of whom complained of more than one symptom) follows:

Symptoms	Number affected
Constipation	10
Giddiness	7
Skin rash	4
Anorexia	4
Fatigue	2
Epigastric pain	1
Profuse sweating	1

Constipation was the most frequent complaint and, apart from a decrease in the frequency of bowel action, patients stated that evacuation seemed incomplete and unsatisfying. A few resorted to laxatives, but in the majority the constipation declined gradually.

Giddiness or dizziness was a not infrequent complaint soon after therapy was started. The symptom was not accompanied by others referable to

hypoglycemia it was not dispelled by food and there was no hypoglycemia found upon blood sugar analysis. There was some suggestion that the size of the initial drug dosage contributed to the occurrence of the dizziness.

Of the 4 patients in whom skin rashes developed (Nos 32, 34, 35 and 46) J had scattered symmetrical mildly itching erythematous eruption which took about 5 to 7 days to subside. This erythematous rash did not reappear in the same patient even when the drug dosage was increased subsequently. In the fourth patient the rash was purpuric and fairly profuse and a thrombocyte count done 3 to 4 days after the purpuric spots and patches appeared registered 180 000 per cubic millimeter of blood. This rash also subsided gradually without reappearance.

*Hematology and urinalysis* There was no appreciable change in the RBC and WBC counts in the patients during treatment with chlorpropamide. Thrombocyte counts were done on 45 patients at intervals in 8 patients (22.2 per cent) the counts registered values at and below 100 000/cu mm of blood but none suffered from bleeding. The majority comprising 31 cases (68.9 per cent) had counts ranging from 100 000 to 200 000/cu mm of blood. The remaining 6 cases (17.8 per cent) had counts higher than 200 000/cu mm. It seems that chlorpropamide can produce a fairly persistent reduction in the thrombocyte count although in the single patient who had a purpuric rash (No 34) the count was 180 000/cu mm of blood.

No abnormality was found when the urine of patients on chlorpropamide therapy was repeatedly examined at intervals for albumin, RBC, WBC casts, and crystals.

### Conclusions

Chlorpropamide is an effective hypoglycemic agent and in this respect it produced good control of the diabetes in 73.1 per cent of the cases studied.

Chlorpropamide is as active in the younger patients (31 to 35 years) as in the older (56 to 60 years).

There is some evidence that the drug is more effective in male diabetics than in female diabetics.

The hypoglycemic action of chlorpropamide is the same regardless of the racial characteristics of the patients studied.

Weight for weight chlorpropamide is a more efficient hypoglycemic drug than tolbutamide 0.66 gm of chlorpropamide being required for maintenance of good control as against 2 gm of tolbutamide.

There is no indication that to effect good control of the diabetes chlorpropamide dosage need be proportional to the height of the fasting blood sugar level.

The maintenance dosage of chlorpropamide is between 0.5 and 0.75 gm per day.

In this study of patients with sthenic plethoric bodily configuration, chlorpropamide treatment yielded a higher percentage and better quality of control of the diabetes than did insulin therapy.

Chlorpropamide may produce undesirable side effects which are frequently mild and subside spontaneously.



There is evidence that chlorpropamide has a mild to moderate thrombocytopenic action

*Acknowledgment*

I thank Government Office of Medical Services, Singapore, for permission to publish this paper, and Chas Pfizer & Co, Brooklyn, N Y for supplying the chlorpropamide used in this trial

*References*

- TAN B Y & R WELLS 1957 A clinical trial of tolbutamide in 220 diabetics Proc Alumni Assoc Malaya 10 240  
WELLS, R & B Y TAN 1957 A clinical trial of sulphonylbutylcarbamide in 51 diabetics Proc Alumni Assoc Malaya 10 124

# CLINICOTHERAPEUTIC EVALUATION OF CHLORPROPAMIDE A NEW SULFONYLUREA DERIVATIVE IN THE TREATMENT OF DIABETES MELLITUS

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Early pharmacological work indicated that chlorpropamide had a higher potency than other sulfonylurea derivatives namely carbutamide and tolbutamide. The objective of the study reported here was to confirm such findings from a clinical point of view in human patients with diabetes mellitus.

The diabetic patients in whom chlorpropamide was assayed were laborers of the National Railways of Mexico; almost all were traveling workers and for that reason required ambulatory treatment. Only patients with uncomplicated diabetes were chosen and none was alcoholic. Selection was made regardless of the activity of the diabetic process, onset of the disease, age, sex, or constitution of the patient. This was done in order to compare the results with those reported in using the earlier antidiabetic drugs which had proved suitable for treating overweight, middle-aged patients who had mild or moderately active diabetes and were free to develop acidosis.<sup>1,2,10,11</sup>

Periodic control during the treatment was performed in 33 subjects for 2 to 6 months; control was observed by daily glycosuria tests (3 times a day) and routine clinical and laboratory examinations. Of the 33 cases studied, 19 were submitted to special weekly laboratory tests including urine analysis, blood chemistry, RBC, WBC, and liver function tests. These tests were chosen in order to detect changes reported as occurring during treatment with carbutamide or tolbutamide (such changes as a decrease in hemoglobin concentration, agranulocytosis, and alterations of the RBC or WBC as described in experimental and clinical works).<sup>6,7,12</sup> Liver damage is probably the most important complication of the treatment with oral antidiabetic drugs;<sup>7,8</sup> for this reason, in several patients a battery of liver function tests was done including thymol turbidity, thymol flocculation, cephalin flocculation, and serum bilirubin. The routine blood chemistry tests included those for glucose, urea, and cholesterol, as well as a glucose tolerance test, which was done in some patients.

The amount of chlorpropamide to be given to each patient was calculated as follows. When no previous treatment had been given, 1 gm of the drug was allowed in patients with glycemia from 250 to 350 mg per cent; when insulin had been administered, 40 to 60 U were considered equivalent to 1 gm of chlorpropamide; when oral sulfonylureas constituted the previous treatment, 1 gm of chlorpropamide was administered instead of 1.5 gm of the other drug. Chlorpropamide was used at a dosage of 1.5 gm only in patients who had been treated with more than 60 U of insulin daily or when a combination of insulin plus oral hypoglycemic drugs was necessary.

The evaluation of the results was made according to the grade of the diabetic process as well as by comparison of the laboratory analyses performed before and after treatment with chlorpropamide

### *Clinical Results*

The clinical results were classified excellent when complete insulin substitution was achieved with satisfactory control and no observed complications good when satisfactory control was achieved without complete insulin substitution or when temporary intolerance was observed and unsatisfactory when a persistent intolerance was present or when insulin requirements could not be decreased

Of the 33 patients (2 females and 31 males 33 to 69 years old) only 5 had severe diabetes and 3 were underweight (TABLE 1) Twenty were designated as giving excellent results 10 good results and only 3 unsatisfactory results

The hypoglycemic effect of the drugs was observed in every patient 2 had hypoglycemia (demonstrated by blood glucose determination) and many showed temporary intolerance especially at the beginning of the treatment The latter symptoms which disappeared with decrease of the drug dosage were probably due to an early and strong hypoglycemic effect In the former 2 cases of hypoglycemic precoma glucose was administered intravenously but the release of the symptoms was observed only after the drug had been discontinued several hours

The final maintaining doses were 125 mg in 4 patients 250 mg in 6 patients 500 mg in 9 patients 750 mg in 1 patient and 1 gm in 10 patients Only in 3 unsatisfactory cases were the doses increased to 2.5 gm a day before discontinuation In 5 patients the drug was discontinued and neither glycosuria nor hyperglycemia has been observed during 1 to 2 months

Insulin was substituted completely in 3 patients in whom it had been the previous treatment in 1 of these patients 250 mg was sufficient to fill the former requirement of 80 U of insulin daily In 9 cases of combined treatment (insulin plus tolbutamide) the complete replacement of insulin was achieved in 7 patients (superseding former insulin requirements of 15 to 40 U daily) with chlorpropamide in the same amount as the former anti-diabetic drugs There were exceptions in 2 cases in which this was reduced still further from 1 gm of tolbutamide to 0.5 gm of chlorpropamide In the other cases of combined treatment the insulin requirement was reduced to 20 U daily maintaining gram for gram the administration of

ie previous medication  
chlorpropamide as tolbut

amide in the other 15 cases the average reduction was from 1.11 gm of other sulfonylureas to 0.445 gm of chlorpropamide which represents an approximate 60 per cent reduction In every case the change from tolbutamide to chlorpropamide was made easily

Of the 20 cases of excellent results almost all were overweight patients and none had severe diabetes Only 2 of the 10 cases with good results had

TABLE I  
RESULTS OF TREATMENT WITH CHLORPROPAMIDE

Excellent										
General data						Previous treatment		Treatment with chlorpropamide		
No.	Age	Sex	Evolu- tion time (years)	Type of diabetes	Con- vul- sion	Insu- lin	Oral anti diabetic drugs	Insulin (U/day)	Chlor- propa- mide (gm/day)	Duration of treat- ment (months)
1	44	M	2	Slight	+10		\$1.00		\$ 500	1
2	49	M	17	Moderate	Normal		\$1.25		\$ 250	2
3	50	M	11	Moderate	+10	120	1.00		1 000	2
4	54	M	4	Moderate	-20		1.00		\$ 125	3+
5	54	F	4	Moderate	+20	30	1.00		1 000	2
6	55	M	1	Slight	+20				\$ 125	1+
7	55	M	5	Slight	+20		1.00		\$ 500	3
8	56	M	22	Moderate	+20	30			\$ 500	1
9	57	M	1	Moderate	+10		\$1.00		\$ 125	6+
10	57	M	4	Moderate	+10		\$1.50		1 000	1
11	60	M	1	Moderate	+10		\$ 30		\$ 250	2
12	61	M	6	Moderate	+20	130	1.00		1 000	1
13	62	M	5	Moderate	Normal	20	1.00		\$ 500	1
14	62	M	6	Moderate	Normal		\$1.00		\$ 125	3+
15	63	M	4	Moderate	+10	40	1.00		1 000	2
16	63	M	5	Slight	+30		1.50		1 000	1
17	63	M	6	Moderate	+20	130	1.00		1 000	1
18	63	M	6	Moderate	+20	30			\$ 500	2
19	66	F	10	Slight	+20		\$1.00		\$ 250	1
20	69	M	19	Moderate	Normal		1.00		\$ 500	2

Satisfactory										
21	58	M	3	Moderate	Normal		\$1.50		\$ 250	2
22	52	M	4	Moderate	+20	140			\$ 250	3
23	54	M	4	Moderate	+20		\$2.00		\$ 500	1
24	58	M	18	Slight	Normal				\$ 750	1
25	59	M	14	Severe	Normal	40	1.00	20	1 000	6
26	60	M	4	Moderate	Normal		\$1.00		1 000	1
27	62	M	5	Severe	+20	160	1.00	40	1 000	2
28	62	M	20	Moderate	+10		\$1.00		\$ 500	1
29	63	M	13	Moderate	+20	115	1.00		\$ 500	6+
30	68	M	22	Moderate	Normal		\$0.50		\$ 250	2

Unsatisfactory										
31	33	M	8	Severe	-20	160		30	2 500	1
32	52	M	12	Severe	-20	4)		40	1 500	1
33	63	M	2	Severe	+10	60		60	1 500	1

severe diabetes and all had a normal weight or were overweight. The 3 cases of unsatisfactory results had been previously controlled with 80 U requirements with as much as 120 U (2, one was underweight, 1, old), and both were psychopathic. These last 2 cases showed persistent

gastrointestinal intolerance to the drug, and no reduction of the insulin requirements could be achieved.

The side effects observed during the treatment with chlorpropamide were almost always moderate, with only transitory intolerances, probably due to overdoses of the drug. Gastrointestinal disturbances such as hiccough, gastric pain, or diarrhea were present in six cases and were of important intensity in only two. In every patient the symptoms disappeared with the reduction or withdrawal of the drug. Nervous system disturbances such as nausea or vertigo, appeared in two cases, disappearing with the temporary discontinuation of the drug. There was only one case of dermatological reaction: this consisted of papula on the head of the patient and was a moderate reaction lasting a short time and disappearing with the reduction of the drug dosage. Three other patients (one not included in these thirty-three cases) observed a generalized peripheral vasodilatation, especially over the face and neck. This reaction, of variable intensity, was originated by the ingestion of a cup of wine or glass of beer, the test was made with other patients, but the effect could not be reproduced.

#### *Laboratory Data*

Unimportant changes were detected by routine urine analysis, no hematuria, cells or casts appeared during administration of the drug. A slight albuminuria was present in some of the patients; this did not increase significantly in any case and in some, was even lower than in the initial control period of the study. This effect was probably due to a better control of the altered metabolic situation.

A decrease in red blood cells observed in 7 patients was less than 1 million in each of 6 and, in one case, the decrease was from 5,100,000 to 4,100,000. Macrocytic hypochromic anemia was present in 2 patients before treatment and did not show any modification during the time the drug was given, 5 of the 7 patients mentioned showed a tendency to hypochromic anemia. In the rest of the cases, the RBC was practically unchanged. No important change was found in the WBC or in the total leukocyte or differential counts.

Total cholesterol was normal or slightly increased before treatment. No important modification could be found in this determination. Blood urea, slightly increased (37 to 44 mg per cent) in 6 cases, was normal, after treatment in every case except 3 of the previous 6 mentioned, in these a slight further increase was found (44 to 55 mg per cent).

One to 3 liver function tests were performed before and after treatment on each of the 19 subjects. thymol turbidity was done in 16 cases, cephalin flocculation in 15, serum bilirubin in 11 and a Bromsulphalein test in 3. No damage was demonstrated by these liver function tests. In 7 cases in which thymol turbidity was slightly increased before the treatment with chlorpropamide (from 6 to 14 U), it was found to be within the normal values after treatment.

In the two cases in which glucose tolerance tests were done, the general morphology of the test was practically unchanged before and after treatment.

### Conclusions

The new compound chlorpropamide showed its hypoglycemic effect in every case. Excellent or satisfactory results were observed in 28 cases of moderate or slight diabetes; unsatisfactory results were reported for 3 patients with severe diabetes and 2 cases of severe diabetes gave satisfactory results. The indications and contraindications of this drug seem to be the same that have been reported for carbutamide and tolbutamide.<sup>1</sup>

The toxic effects seem to be similar to those reported for the other anti-diabetic drugs; the observed lack of response to glucose administration was similar to that reported in animals by Houssey.<sup>19</sup>

Although these drugs seem to have no effect on the metabolism of alcohol,<sup>18</sup> the ingestion of it produced important peripheral vasodilatation in three cases; this effect was not accompanied by hypertension or headache and lasted for two to four hours.

No toxic effect could be shown by the liver function tests; such findings are difficult to evaluate because of the liver dysfunction that is frequently present in treated or untreated cases of diabetes mellitus.<sup>17, 19</sup>

The potency of the drug seems to be 2 to 3 times that of carbutamide or tolbutamide but has individual variation from case to case; some patients are well controlled with 0.125 gm. of chlorpropamide while others require as much as 1 or 1.5 gm. in order to achieve correct control.

### References

1. GOLDNER M G & WEINSKY 1958 N Y State J Med 68(5) 665
2. LEVIN R & G G DUNCAN 1956 Metabolism 5 771
3. SHERPHEVLY N A 1958 Abstr J Am Med Assoc 168(5)
4. DUNCAN G G C T LEE & J K YOUNG 1957 Ann N Y Acad Sci 71(1) 233
5. SEIDLER I W FIDRES & R SPIS 1957 Deut med Wochschr 82 1518-1524
6. ANDERSON R C H V NORTH & P N HARRIS 1957 Diabetes 6(2) 146
7. MARBLE A & R CAMERINI D'ALLOS 1957 Ann N Y Acad Sci 71(1) 9
8. FIELD J B D D FEDERMAN 1957 Diabetes 6 67
9. SCHAMBEY P 1957 Diabetes 6(2) 146
10. SHERRY S J L ZILFFREN S R BRAISERMAN & A W DREW 1957 Ann N Y Acad Sci 71(1) 241
11. PURVELL R A ARAI E PRATT C HLAND JR & H ELRICA 1956 Metabolism 5 773
12. CAMPBELL J 1957 Can Med Assoc J 74 962
13. SIREK A M O V SIREK & C H BEST 1957 Diabetes 6(2) 151
14. LOUBATIERES A J 1957 Diabetes 6 408
15. HOLDSAY B A & J C LEVINGS 1956 Metabolism 5 727
16. FARNEY R J & H R HULPIEU 1957 Diabetes 6 78
17. PROMENAZ J 1951 Bull N Y Acad Med 27 396
18. ZIMMERMAN H J F G McMURRAY H RAFFAPORO & L K ALPERT 1939 J Lab Clin Med 112 7
19. LEEVY C M C M RYAN & J C FINETBERG 1950 Am J Med 11 290

# CLINICAL STUDIES WITH CHLORPROPAMIDE

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Fifteen office patients with diabetes were treated with chlorpropamide. In 6 it was necessary to discontinue the drug: 4 developed ketosis, 2 lost considerable weight and had poor appetites, although their blood sugars approached normal values and there was no glycosuria, 9 are still under observation. The shortest period of follow up was 2 months, the longest, 6. The ages of the 4 men and 5 women treated ranged from 35 to 70. All but 1 are diabetics who had been on insulin and who, at other times, managed their diabetes by dietary means, thus providing the factors necessary for a comparison.

All of these patients were permitted a normal or usual diet. They neither weighed nor measured their food, and they had sweets on occasion. At first they were seen every other day, then at weekly intervals and now at monthly visits. The dosage of chlorpropamide varied from 500 to 1000 mg. At each visit the patients were questioned as to symptoms of diabetes or hypoglycemia (or both), pruritus, skin eruptions, or weakness, later they were also questioned as to loss of appetite. Body weights were recorded, and blood glucose levels were determined. Urine was examined for ketones and glucose. Blood counts were obtained.

At first the immediate response to the drug was tested. The 5 patients used were 30 to 60 years old, and had had diabetes from 1 month to 14 years. All came to the office without breakfast and, after blood was drawn and a urine specimen obtained, 1 gm. of chlorpropamide was administered to each. One hour later, blood and urine samples were obtained, and the patients were then sent out for a breakfast consisting of fruit juice, 2 eggs, 1 slice of toast, and a beverage, some had cereal instead of the eggs. The blood and urines were examined two hours after this breakfast, and a fasting specimen was taken 24 hours after the initial administration of the drug.

From TABLE 1 it is clear that only 2 of the 5 unselected patients showed a hypoglycemic reaction to the test dose. Patient W. M. showed a consistent fall in the blood glucose, the effect persisting overnight. The chlorpropamide blood levels were constant, and only a slight drop was seen after 24 hours. With so definite a response, this patient appeared suitable for therapy with chlorpropamide. However, since he was obese and his diabetes was of only 1 month's duration, he was treated by subcaloric diet only, no drug being given. He lost weight slowly and, with this weight reduction, his glycosuria cleared. He has no symptoms and his last fasting blood sugar was 130 mg. per cent. Had we given him chlorpropamide with the prescribed diet, we certainly would have considered the result a successful

\* Chlorpropamide levels were determined by Chas. Pfizer & Co. Inc., Brooklyn, N. Y.

response to the drug. Patient P. B. has had diabetes for 4 years. His symptoms were inconstant and his glycosuria although occasional was most apparent postprandially. He weighed 215 lb and 4 years ago dietary therapy alone was advised. On a subcaloric diet he lost weight with the loss of 30 lb. however he also had symptoms of diabetes with intense fatigue as the most troublesome feature. He refused insulin and chlorpropamide seemed ideal for him. His hypoglycemic response showed the test dose to be a failure even though the drug's blood level was sustained. His firm refusal to use insulin however prompted us to try chlorpropamide. He

TABLE I  
DATA ON SIX PATIENTS TREATED WITH CHLORPROPAMIDE\*

Patient	Sex	Age	Duration of diabetes	VPH fasting	Blood sugar mg per cent*				Chlorpropamide blood levels $\mu\text{g/ml}$			
					Fasting	1 hour after 10 gm dose	2 hours after breakfast	After 24 hours	Fasting	After 1 hour	After 3 hours	After 24 hours
W. H.	M	50+	1 month	0	225 S + +	192 S +	148 S +	154 S 0	0	04	07	III
R. B.	M	50+	4 years	0	308 S + +	204 S + +	186 S + +	165 S + +	0	8	03	III
H. J.	M	40+	3 years	10	210 S +	180 S +	104 S 0	153 S +				
J. D.	F	30+	10 years	50	130 S + + AC +	330 S + + AC 3 +	440 S + + AC 4 +	Yemenated R25	0	46	64	—
S. R.	M	60	14 years	60	332 S + + AC 0	322 S + + AC 4 +	295 S + + AC 4 +		0	35	24	397 504
E. C.	F	60	2 years	0	360 S + +		360 S + +					

\* Lower figures or figures S represents urine sugar AC ketones

† Results are for same sample as

was given 10 gm daily surprisingly the glycosuria cleared most of his symptoms of diabetes disappeared and for 2 months his fasting blood sugars ranged from 170 to 200 mg per cent. The liver function tests were normal as was the hemogram. The chlorpropamide level was 318  $\mu\text{g/ml}$ . All of this was spectacular but although he ate well he did not enjoy his food and was losing weight at the rate of 5 lb per month. Because we suspected some unpleasant complication he was hospitalized but an extensive study failed to reveal any abnormalities. While he was in the hospital the chlorpropamide was continued and he chose his own foods. Although all fractional urine specimens were sugar free and the blood sugars were 125 and 132 mg per cent he felt tired did not enjoy his food and failed to gain weight even though he forced himself to eat his meals. Failure to improve



## THE USE OF CHLORPROPAMIDE IN BRITTLE AND POORLY CONTROLLED DIABETES MELLITUS\*

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The development of the oral hypoglycemic agents has led to some sweeping revisions in concepts about the control of the abnormal carbohydrate metabolism in diabetes mellitus. With the compounds presently available (tolbutamide in the United States and oral therapy is available only in diabetes<sup>1,2</sup>). It is well known that diet alone or by diet with small doses of insulin. Consequently use of the oral agents for these patients has been more a convenience than a necessity. Patients in urgent need of some agent that will provide more stable control are the juvenile diabetics and the adult diabetic patients who are poorly controlled on any dose of insulin. Included in the latter group are those patients who are brittle in the sense that there is irregular daily alternation between gross hyperglycemia and overt or occult hypoglycemia. In these patients many factors are involved such as poor diet, irregular habits, subcutaneous reactions at the sites of insulin injection, insulin overdosage, inability to break down complex insulin, and infection—to name only a few. As a rule tolbutamide and carbutamide are ineffective in such patients although an occasional excellent response has led to the hope that some other sulfonylurea would be helpful in achieving significantly improved clinical control.

With the development of chlorpropamide which was thought to be a more potent agent it was felt that an adequate trial in the brittle, unstable, and poorly controlled diabetic was justified. Patients were selected from the Diabetic Clinic at Jefferson Davis Hospital and referred to a special clinic for oral therapy. The patients attending the diabetic clinic at this hospital have been characterized in detail in the literature.<sup>3</sup> In general they are in poor financial circumstances, of low intelligence and poorly educated. Most are obese and urinary tract infection is a major problem.

Chlorpropamide was started in large doses (as much as 1.5 gm. per day) in the poorly controlled diabetic receiving large doses of insulin. In general the patient's insulin dosage was reduced by one half when chlorpropamide therapy was instituted. This was the usual practice if the patient was receiving more than 50 U of insulin. Those with mild stable diabetes received 500 to 1000 mg per day as the initial dose and insulin was discontinued. Treatment was carried out on an outpatient basis and the

\* The work reported in this paper was supported by a grant from Chas. Pfizer & Co. Inc. Brooklyn N. Y.

† Trainees in Diabetes Training Grant Program National Institute of Arthritis and Metabolic Diseases Public Health Service Bethesda Md

patients were provided with explicit instructions to communicate with one of the physicians on the metabolic service if anything unusual occurred. No additional instruction in dietetics was given to any of these patients; this was done deliberately to obviate the possibility that any degree of improved control would result from improved dietary habits. The patients were checked at weekly intervals until they had become stabilized on the oral therapy, the period between visits was then lengthened. Doses of insulin and chlorpropamide were altered as deemed advisable from the clinical course. White blood counts, differentials, blood urea nitrogens, thymol turbidity, blood sugar and chlorpropamide blood levels were determined at each visit. The patients who missed appointments were asked to appear later. Sixty-one patients have received therapy for a time sufficient to allow evaluation.

### Results

The status of the sixty one patients is summarized in table 1. Three patients died while receiving chlorpropamide therapy. One of these died

TABLE 1  
STATUS OF PATIENTS OBSERVED IN STUDY

Deaths since chlorpropamide therapy instituted		3
Not related to drug	2	
Related to drug	1	
Received therapy for 1 week for special metabolic study		3
Drug discontinued because of increasing Hb A <sub>1c</sub> symptoms		4
Restarted on drug therapy	2	
Lost to follow up		4
Uncooperative; drug therapy discontinued		6
Toxic reactions; drug discontinued		12
Withdrawn because of poor control		1
Receiving uninterrupted drug therapy		28
Total		61
Presently on therapy		30

of what appeared to be a myocardial infarction following a typical allergic reaction to penicillin. Chlorpropamide therapy had been discontinued one week before death and could be connected in no way with the death. The second was a patient with advanced generalized arterio-sclerosis who developed an extremely severe urinary tract infection and died of septicemia. It was felt that chlorpropamide therapy was not involved in this patient's demise. The death of the third patient was felt to be related to the chlorpropamide therapy.

### Case Reports

S. K. was a 61 year old white female who was diagnosed as having mild diabetes mellitus in 1954. She had a marked aversion to injections and refused to take insulin. Control was good but with minor stress she became

poorly controlled. She requested oral therapy and was started on 500 mg of chlorpropamide daily in a divided dose. Approximately 2 weeks later she became ill and was treated by a private physician who made a diagnosis of respiratory tract infection. The patient was given Azotrex which contains tetracycline, sulfamethazole and Pyridium. The physician found no evidence of hepatitis at the time of his examination. Her symptoms became more acute and 4 days later she was admitted to Jefferson Davis Hospital suffering with nausea, vomiting, dehydration and jaundice. She rapidly developed electrolyte disturbances and congestive heart failure and expired. At autopsy there was hepatitis of the cholestatic type most commonly seen with chlorpropamide idiosyncrasy. This case will be reported in detail elsewhere. It is felt that had this patient reported to the clinic at the time she became ill as she had been instructed, she probably would have survived since she continued on sulfonylurea therapy from the onset of symptoms until admission to the hospital.

Significant improvement in controls was achieved in four patients but the drug was discontinued because of an increasing level of blood urea nitrogen accompanied by symptoms of malaise, weakness and lethargy. It was originally felt that this might be a consequence of drug therapy but it is now felt that an acute flare up of chronic urinary tract infection caused most of the symptoms. Two of the four patients have received chlorpropamide therapy again with no untoward effect. Six patients were lost to follow up.

Patients who had been actively uncooperative in the therapy preceding that with chlorpropamide often continued this erratic behavior after being started on oral therapy. It is our impression that these patients did not do well especially when judged by a sense of well being, general appearance and clinical course. Often their chemical control was quite good. Chlorpropamide blood levels fluctuated quite widely, probably indicating that they had taken their tablets erratically. It is felt that patients who continue uncooperative and erratic should not be continued on this form of therapy. Toxic reactions of such severity as to force abandonment of chlorpropamide therapy occurred in 12 patients: 2 showing somnolence, 4 dermatitis and 6 gastrointestinal symptoms.

It was necessary for the families of 2 patients with somnolence to go to extremes to awaken them to receive food and medicine. One patient who developed a skin rash also experienced mild to moderate somnolence on the drug. Of the 12 patients who discontinued chlorpropamide because of toxic reaction, 6 had received a maximum dose of 1000 to 1500 mg daily and 6 had received a maximum of 250 to 750 mg daily. Of the 32 who had discontinued drug therapy at some time, 10 had received at least 1500 mg of chlorpropamide for days to weeks while of the 30 presently on therapy, 13 have received 1500 mg or more daily for a week or longer. Hypoglycemia was not mentioned in the side effects as this is a natural consequence of the drug therapy with the large doses being given in this study; hypoglycemia was a common phenomenon. A large percentage of all of the patients treated had mild hypoglycemia some time before they were stabilized.

Ten of the patients underwent moderate hypoglycemic episodes while 3 had severe hypoglycemia.

Of the thirty patients still receiving chlorpropamide seventeen are in excellent control, three are in good control and ten are in fair control all are significantly improved relative to their condition during previous therapy.

Of the original 61 patients 33 may be classified as poorly controlled or brittle diabetics. 19 of these are presently receiving chlorpropamide. This agent has proved to be extremely useful in improving the control of the patient who had been uncontrolled on other regimens. Chlorpropamide was used in combination with insulin in 13 of these cases. In 5, however, insulin

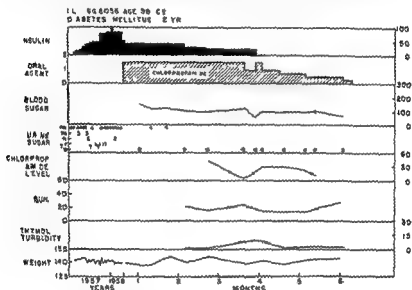


FIGURE 1

was eventually discontinued chlorpropamide was used alone in patients who had received as much as 90 U of insulin. The following case reports illustrate the results obtained.

I. L., a 58-year-old Negro female in whom the diagnosis of diabetes mellitus was made in 1956, was admitted to the hospital in September 1956 with a subacute myocardial infarction and congestive heart failure. She was treated with digitalis, diuretics, bed rest and diet with good results. Her course since that time is shown in FIGURE 1. She developed an almost constant 4+ glycosuria associated with general lethargy and malaise and with occasional episodes of overt hypoglycemia. The response to chlorpropamide has been quite good. Insulin has been discontinued and the patient is maintained on 250 mg of chlorpropamide daily.

H. S. is a 63-year-old Negro female with diabetes mellitus of 22 years' duration. When first seen at the hospital in 1939 she had a urinary tract

infection associated with chronic cervicitis. In 1944 she was treated for a Bartholin abscess and, in 1953, she was found to have a *peripheral neuropathy* and atypical arthritis. In 1957 a diagnosis of arteriosclerotic heart disease with *auricular fibrillation* and *congestive heart failure* was made. There was a satisfactory response to digitalis, diuretics, and salt restriction. This patient has a seasonal job and finds it difficult to obtain adequate food when unemployed in the summer. When this occurs, she discontinues her hypoglycemic therapy, as shown in FIGURE 2. She is now receiving 500 mg chlorpropamide daily, which provides more adequate control than as much as 100 U of insulin. This patient was hospitalized on August 26, 1958, and

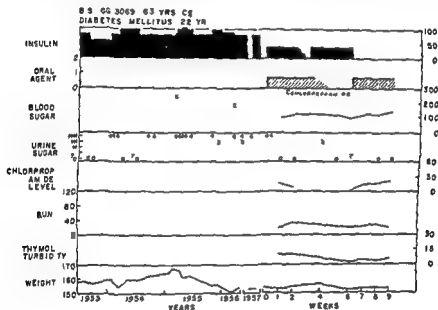


FIGURE 2

a small granuloma of the lung resected. She was treated with chlorpropamide throughout her hospital stay, with the exception of the day after surgery, on which she received no hypoglycemic therapy. In the immediate postoperative period moderate glycosuria appeared, mainly while she received intravenous therapy. On the third postoperative day she received 30 U crystalline insulin in divided doses. The glycosuria cleared the next day and she was discharged in excellent condition 1 week later. This patient is achieving significantly better control on chlorpropamide alone than she did on insulin alone. In addition, it was possible to carry her

duration, was first seen at Jefferson Davis Hospital in 1951 with uncomplicated diabetes mellitus, mild acidosis, and pyelonephritis. She was admitted

1 month later with a carbuncle on the right buttock acidosis and severe pyelonephritis. In the period 1952 to 1956 she was admitted 5 times with severe urinary tract infection usually associated with acidosis. It was possible to suppress the urinary tract infection but not to eradicate it in spite of all diagnostic and therapeutic procedures. In 1957 she was found to have a peripheral neuropathy and Kimmelsteil Wilson syndrome was

all forms of antibiotic therapy. In spite of the infection it was possible

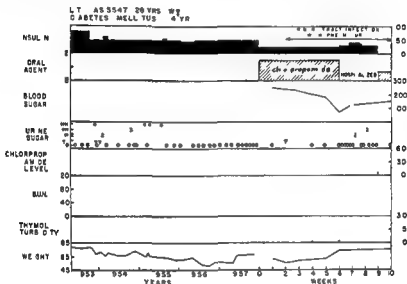


FIGURE 3

to discontinue the use of insulin and to achieve adequate control with 750 mg of chlorpropamide daily. Dissatisfied because of the continued urinary tract infection she did not cooperate with the oral hypoglycemic therapy and began to manifest evidence of poor control. This patient is classified in our uncooperative group of patients. It was evident that as long as she cooperated with therapy her control (both chemical and symptomatic) remained quite good; when she became uncooperative she rapidly developed symptoms that required discontinuation of chlorpropamide and reinstitution of insulin. This patient's history is summarized in FIGURE 3.

■ W, a 30 year old Negro male in whom the diagnosis of diabetes mellitus had been made four years previously, had been hospitalized for two weeks in January 1955 with poor control and acidosis. He had previously failed to respond to carbutamide and tolbutamide. All general manifestations of poor control were present: such symptoms as weight loss, weakness, inability

to carry out sustained effort, and increasing susceptibility to infections. The response to chlorpropamide in this patient has been excellent. As in this patient, it is easy to see that chlorpropamide is effective when others of the sulfonylurea compounds have proved ineffective. This history is shown in FIGURE 4.

P. D., a 55-year-old white female with known diabetes of two years' duration was first seen at the hospital in May 1955 with paroxysmal auricular tachycardia that responded to parenteral digitalis. She was hospitalized for four weeks in December 1956 because of poor diabetic control, angina pectoris and a posterior myocardial infarction of indeterminate age. During

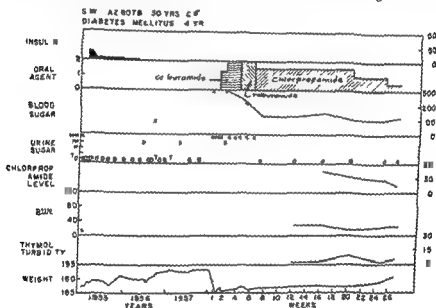


FIGURE 4

this hospitalization tolbutamide therapy was started but was discontinued with the onset of an acute severe urinary tract infection. Later she was treated with carbutamide with fair initial response but secondary failure. Initial insulin therapy produced almost no improvement. When the insulin dosage was increased the patient developed rather severe hypoglycemia at irregular intervals. She developed severe hypoglycemia on the first day of combined therapy of chlorpropamide and insulin. Reduction in the chlorpropamide dosage led to significantly improved control. This patient subsequently left the community, and recent communication with her private physician at her new location gave the information that she has remained in excellent control. Data on this history are in FIGURE 5.

A. N. is a 59-year-old white female whose primary difficulty has been extensive nodule formation at the sites of insulin injection. As a consequence, she has alternating periods of hyper and hypoglycemia that, at

times, have completely disrupted her life. With chlorpropamide therapy, the dose of insulin given has been reduced significantly. The nodule formation has not been a major problem at this reduced dose level of insulin. Details of this history are shown in FIGURE 5.

■ C is a 66 year old white female with diabetes of thirteen years' duration and has been observed for more than twenty years at the Jefferson Davis Hospital. This patient has myxedema and has had surgical removal of a nontoxic benign thyroid adenoma. For several years she has had recurrent acute episodes of a chronic severe urinary tract infection. She has arteriosclerotic heart disease with a left bundle branch block and peripheral neu-

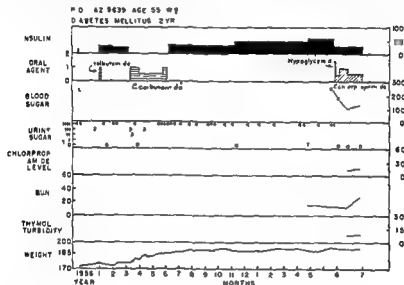


FIGURE 5

ropathy. At no time has this patient's diabetes come under satisfactory chemical control, in spite of all efforts in the Outpatient Clinic, plus multiple hospitalizations. The response to chlorpropamide has been dramatic, however, she continues with urinary tract infections, and the weight loss noted in the chart is due to the continued infection rather than the chlorpropamide therapy. This case is illustrated in FIGURE 7.

L. W., a 70 year-old white male with diabetes mellitus of 20 years' duration, was admitted to Jefferson Davis Hospital in 1950 with acidosis, and in 1953 with the same complaint. In 1957 he was found to have arteriosclerotic heart disease with congestive heart failure, which responded to digitalis, diuretics, and salt restriction. At the time of this hospitalization he also had mild hypertension and bronchopneumonia. He has received as much as 130 U of insulin daily, with a clinical course characterized by alternating hyper- and hypoglycemia. He refuses to take less than 100 U



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DIABETES MELLITUS 18 YR

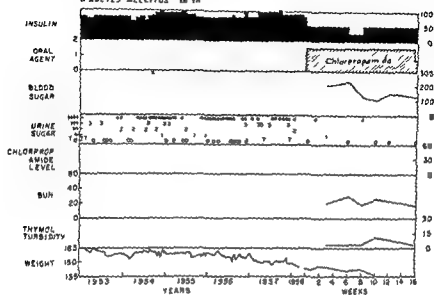


FIGURE 6

BC AF 9980 66 YRS W3  
DIABETES MELLITUS 13 YR

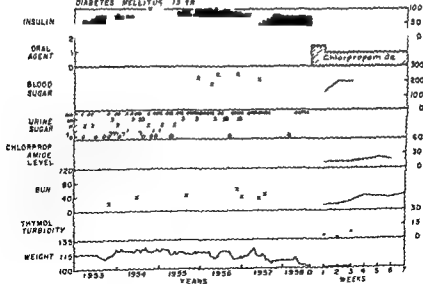


FIGURE 7

of insulin a day however the addition of chlorpropamide to 1500 mg daily has significantly improved his chemical control. His clinical course is much smoother as he does not alternate between hyper and hypoglycemia and feels much better than he has for many years. Details are shown in FIGURE 8.

J J is a 35 year old Negro female with diabetes mellitus of 7 years duration. At onset her diabetes was relatively mild and comparatively easily controlled. In the past two years however she has presented one of the most unusual and bizarre problems in diabetic control that has ever been seen in this clinic. She has developed extensive nodule formation at the sites of insulin injections and in addition approximately once a month

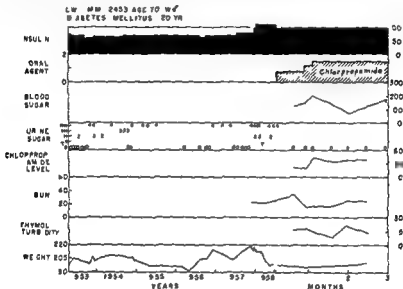


FIGURE 8

develops a delayed hypersensitivity response at the sites of injection. During these two years it has never been possible to achieve significantly improved control in spite of the fact that it has been possible to put her into an environment that is well controlled where she receives meals regularly and where nutritional deficiencies are corrected. She was first started on DBI. By chemical criteria this produced only minimal improvement in control by clinical criteria however the results were quite striking. It was then felt desirable to change from DBI to chlorpropamide to determine if any improvement in the chemical control could be achieved. As may be seen in FIGURE 9 there was very little difference in the control achieved by each of these drugs. It should be noted however that she is receiving about 50 per cent of her previous dose of insulin. It is obvious too that occasionally she exhausts her insulin supply and neglects to return to the hospital for an additional supply. As a criterion for the improvement in control in this patient it

may be stated that in the  $1\frac{1}{2}$  years prior to the institution of oral therapy, this patient had been hospitalized one fourth of the time. Since the institution of oral therapy she has not been hospitalized.

W. A. is a 64 year-old white male with diabetes mellitus of three years' duration. When the diagnosis was first established, he was treated with small doses of insulin. However, he failed to return to the clinic and was not seen again until early in 1958.

Because of the previous good results of chlorpropamide it had been the desire of the metabolic section to attempt to treat a person in acidosis with intravenous chlorpropamide. On admission, this patient was in moderate

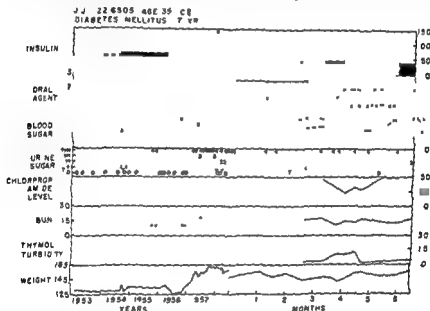


FIGURE 9

acidosis with clinical signs and symptoms, but he was not in coma. He was administered 1000 mg of chlorpropamide intravenously during a 6 hour period. Other fluids were given as is indicated in FIGURE 10. It is evident that the patient's acidosis was relieved promptly. Glucosuria and hyperglycemia continued under the impact of the intravenous glucose. A total of 70 U of insulin was given after the patient was cleared of acetonuria, in an attempt to decrease the glucosuria than to control the acidosis.

moderate acidosis may be treated with cement, without the administration of insulin. The patient indicates that the use of cement may be worth further exploration.

As may be seen practically all the patients received large doses of chlorpropamide for weeks to months. They tolerated these large doses quite well, and it is our impression that the only toxic manifestations that occurs

more frequently in this group of patients are nausea and vomiting. It appears then that the poorly controlled diabetic receiving large doses of insulin will tolerate large doses of chlorpropamide for long periods of time without manifesting overt toxicity.

The term diabetic control has many connotations to many people. Control may be spoken of as clinical or chemical with subdivisions as far as glycosuria, hyperglycemia, weight gain, sense of well being, and other criteria are concerned. In the 30 patients who are presently on therapy, an attempt was made to subdivide diabetic control into control of glycosuria, control of blood sugar, control as judged by weight gain or loss, and control as

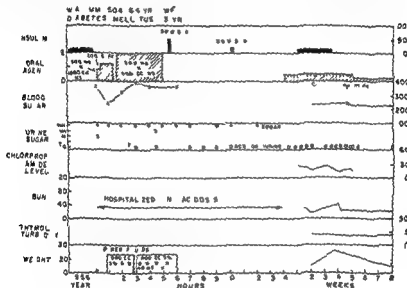


FIGURE 10

judged by general clinical course. Degrees of control in each category were expressed as excellent, good, fair, poor, and uncontrolled. As judged on the basis of glycosuria, control was excellent in 20, good in 3, fair in 5, and poor in 2. Based on maintenance of a satisfactory postprandial blood sugar, excellent control was achieved in 14, good in 7, fair in 6, and poor in 3. On the basis of weight, 25 were in excellent control, 3 good, 1 fair, and 1 poor. Symptomatically, 26 were in excellent control, 3 good, and 1 fair. It is noteworthy that the drug was discontinued when patients were uncontrolled or in poor control symptomatically.

In addition to the brittle and poorly controlled diabetics reported above, there were 8 patients in whom the diagnosis of diabetes mellitus had been made recently. All of these were of the type that needed some hypoglycemic therapy and chlorpropamide was administered. Of these 8, 7 are still

receiving oral therapy and only one is receiving this in combination with insulin

### Summary

Chlorpropamide has proved to be a valuable adjunct to the therapy of brittle and poorly controlled diabetics. In patients receiving large doses of insulin it has been possible in most instances to reduce the dose of insulin by at least 50 per cent and to achieve a significantly smoother and more stable course for the patient. One case of toxic hepatitis with death is reported apparently this was due to idiosyncrasy to the drug. Chlorpropamide remains effective under such stress situations as infection and surgical operation and on the basis of one case in the presence of acidosis.

### References

- 1 MOORHOUSE J A & R M LARA 1956 Physiologic actions of Orinase and their relationship to the types of diabetes in man *Metabolism* 5 847
- 2 COX R W E D HENLEY E B FERGUS & R H WILLIAMS 1956 Sulfonylureas and diabetes mellitus I Clinical evaluation *Diabetes* 5 358
- 3 PANEL DISCUSSION 1958 Oral hypoglycemic agents *Diabetes* 7 53
- 4 DONSON H L H S LIPSCOMB J A GREENE & H T ENGELHARDT 1958 Socio-economic status and diabetes mellitus *J Chronic Diseases* 7 413

### Discussion of the Paper

GARFIELD G DUNCAN (Philadelphia Pa.) I have three patients who became refractory to tolbutamide therapy and later required a larger dose of insulin than previously. Have any of the investigators in basic research repeated the experiments of Allen who performed a partial pancreatectomy in dogs to produce a state just short of diabetes and then provoked diabetes by overfeeding thus stimulating the islets of Langerhans?

It seems to me that there may exist a similarity between these experiments and experiences with the patients who require more insulin than before sulfonylurea treatment. I suggest that we do the same thing and try to precipitate diabetes in such partially pancreatectomized animals with the sulfonylurea compounds. Although this may have been done I have not heard of it.

MARTIN G GOLDNER (Jewish Chronic Disease Hospital Brooklyn N Y) The question whether secondary failures to sulfonylureas have an increased insulin requirement is an important one. From what I have seen and from what I know of the literature Lamar's experience does not appear to be the rule. I recall for instance Pfeiffer's<sup>1</sup> series of thirty secondary failures only three or four of these had an increased insulin requirement and the increase was rather small while all others required the same amount or even less than that needed prior to sulfonylurea treatment. However one must realize that there are at least three different types of so called secondary sulfonylurea failures: there is first the patient who overeats and relaxes his diet when placed on the drug; second the true secondary failure who without any detectable cause escapes from control; and third the patient in whom the diabetes itself has become worse. We must not forget that we deal with a disease that may be stable for some time but that may also progress at any

time. It would not be surprising if the first or the third type need more insulin but neither would indicate true failure of the sulfonylureas. The first is a failure of dietary management the third an idiopathic aggravation of the disease. Only the second group can be linked causally to the hypoglycemic drugs and in this type the general experience seems to be that the insulin requirement is practically unchanged.

# *Reference*

1. PIZZIERE F. 1957. *J. Endocrinol.* 15: 48.

# ADVANTAGES AND DISADVANTAGES IN SHIFTING PATIENTS FROM TOLBUTAMIDE TO CHLORPROPAMIDE

J. Bloch and A. Lenhardt

*Metabolic Disturbances Service, Krankenhaus der Stadt Wien Lainz, Vienna, Austria*

Because of the chronicity of diabetes mellitus, permanent continuous treatment is necessary. Therefore, efforts have been made (and continue to be made) to develop new clinically effective agents that have pronounced hypoglycemic effect. It is most desirable that minimal dosage of such agents produce an optimal antidiabetic effect and, further, that the lowest possible incidence of untoward side reactions be associated with therapy. In keeping with this research objective, the Austrian branch of Pfizer International Inc., New York, N. Y., supplied us with the new antidiabetic drug, chlorpropamide, for clinical evaluation.

## Materials and Methods

Two hundred inpatients were treated with chlorpropamide, special consideration being given to evaluating the clinical advantages and disadvantages of the drug in comparison with those of tolbutamide and carbutamide.

TABLE I  
DATA ON DIABETICS SUCCESSFULLY CONTROLLED WITH CHLORPROPAMIDE

Previous measures	No. of patients	No. with good control on chlorpropamide only
Diet only	45	43
Tolbutamide and carbutamide	26	20
Insulin and introductory chlorpropamide	56	40
Prolonged insulin	73	43
Total	200	146

More than 1700 diabetic patients have been treated with the latter 2 preparations for a period of almost 3 years. In addition, 15 outpatients were put on chlorpropamide during our clinical investigation.

In July 1958 we were able to report on 136 patients studied to that time. We have now enlarged this group to 200 patients who either had been on insulin or whose diabetes had not been satisfactorily controlled by dietary restriction alone, even though they were hospitalized. The above figure also includes those patients who had been treated with tolbutamide, carbut-

insulin, the remaining 83 had been . . . Of the latter, 40 received insulin only . . .

admission to the hospital in order to stabilize their diabetic condition rapidly the remaining 43 had been controlled with insulin for more than 10 years

The protracted therapy employed for periods as long as 5 months (follow-ups were carried out at 1 week intervals) brought about very good metabolic equilibrium in 25 of 38 cases and just sufficient control in 5 cases. In the latter fasting blood sugar was about 200 mg per cent and 24 hour glycosurias were up to 15 gm. In the remaining 8 patients control with chlorpropamide was discontinued because of increasing glycosuria which obviously

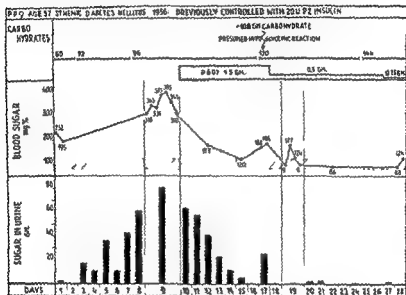


FIGURE 1 Chlorpropamide induced increase in carbohydrate tolerance

resulted from neglect of the strict dietary rules. Acidotic and pregnant diabetics were not treated with chlorpropamide. The usual complications for example retinopathy, sclerosis of the coronary arteries, nephropathy and obliterating arteriosclerosis of the extremities do not constitute contraindications for chlorpropamide treatment. It follows that the control of diabetes with chlorpropamide is more successful—the majority of the patients respond in those in whom diabetes becomes manifest at or after the fifth decade of life. This applies especially to diabetics who previously were controlled by dietary restriction alone (TABLE 1). In such cases chlorpropamide allows a larger intake of carbohydrates while effecting a nearly normal fasting blood sugar, an excellent 24 hour blood sugar profile and sugar free urine (FIGURE 1).

Also evident is the fact that in patients taking insulin, the possibility of success with chlorpropamide decreased as the need for insulin increased. In the majority of cases requiring less than 40 U insulin daily, chlorpropamide



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In July 1958 we were able to report on 136 patients studied to that time. We have now enlarged this group to 200 patients who either had been on insulin or whose diabetes had not been satisfactorily controlled by dietary restriction alone even though they were hospitalized. The above figure also includes those patients who had been treated with tolbutamide, carbutamide or both. TABLE I shows that 146 patients (more than two thirds of the diabetics who appeared suited to oral therapy) could be well controlled with chlorpropamide. Of the 146 patients 63 had not been controlled by insulin; the remaining 83 had been. Of the latter 40 received insulin only on

ups were carried out at 3 week intervals) brought about very good metabolic equilibrium in 23 of 38 cases and just sufficient control in 5 cases. In the latter fasting blood sugar was about 200 mg per cent and 24 hour glycosurias were up to 15 gm. In the remaining 8 patients control with chlorpropamide was discontinued because of increasing glycosuria which obviously

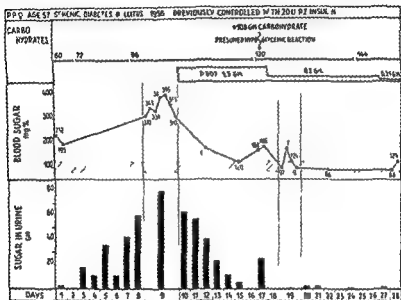


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Also evident is the fact that in patients taking insulin the possibility of success with chlorpropamide decreased as the need for insulin increased. In the majority of cases requiring less than 40 U insulin daily, chlorpropamide

could be substituted with success. However, even in 21 diabetic patients requiring as much as 60 U of insulin daily, we succeeded in achieving good metabolic equilibrium with chlorpropamide. Regarding duration of the disease and, to a lesser extent, duration of insulin medication quite favorable

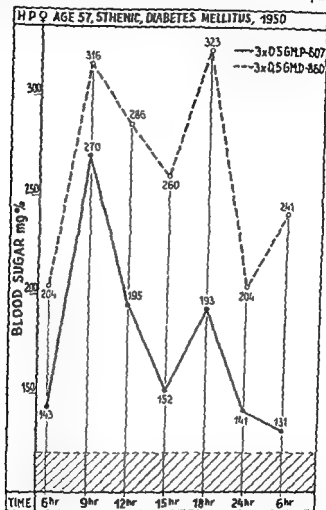


FIGURE 2

exceptions to the 10 and 5 year rule respectively could be obtained with chlorpropamide as was demonstrated in 26 diabetics in whom we replaced insulin completely although they had been receiving insulin for 10 to 20 years. Recent diabetics even those of the asthenic type sometimes did well on chlorpropamide if they were well beyond 40 years of age.

Regarding the responses in the two large diabetic groups the brittle and the stable, it may be stated that, in general the same criteria are applicable

with chlorpropamide as with the oral antidiabetic drugs previously used. However, as with other drugs, exceptions exist and because mixed forms result from the overlapping of the classifications brittle and stable the exceptions increase.

### Observations

The effect of chlorpropamide in reducing blood sugar and sugar in urine is greater than that of the two other oral antidiabetics now in use. Hence

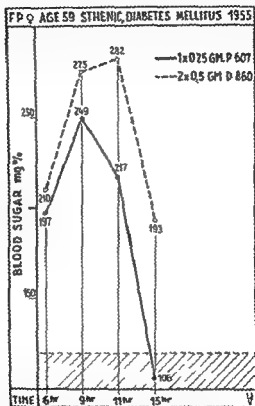


FIGURE 3

with a lower dosage of chlorpropamide (sometimes substantially lower especially during the initial treatment period) equally favorable therapeutic results can be achieved together with good equilibrium of metabolism (FIGURES 2, 4, 4 and 5). The initial daily dosage usually was 1 gm (two 500 mg doses) and in some cases 1.5 gm (three 500 mg doses). Maintenance dosage was usually 0.5 gm or 0.25 gm frequently given every other day. In patients on relatively low quantities of carbohydrate (for example 80 to 120 gm daily) higher initial dosage can lead to sudden hypoglycemic episodes.

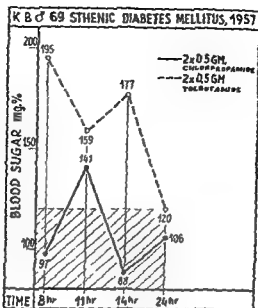


FIGURE 4

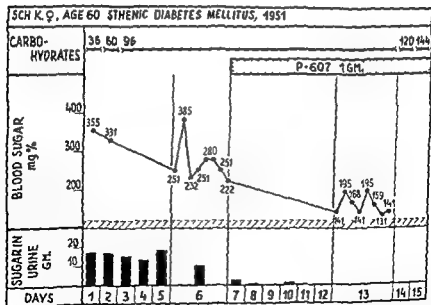


FIGURE 5 Example of good control with chlorpropamide. Patient previously controlled with 20 U lente insulin is now controlled with two 0.5 gm doses carbutamide daily.

especially if the treatment is preceded by a considerably restricted, although short term, diet (48 to 60 gm carbohydrate)

The following episode supports the theory (of the mechanism of action of the oral antidiabetic drugs) that these preparations promote or activate endogenously produced insulin. The dextrose loading test carried out in a nondiabetic (FIGURE 6) who because of obesity was put on a restricted diet (especially with respect to carbohydrates) showed very good endogenous

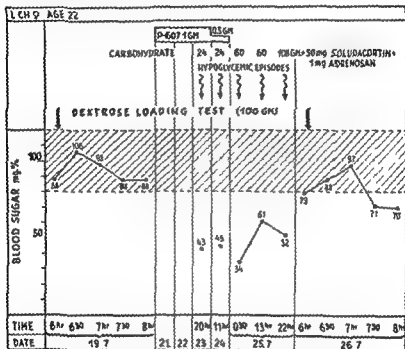


FIGURE 6 Promotion by chlorpropamide of endogenously produced insulin

insulin action. After the administration of six 500 mg doses of chlorpropamide in a period of 3 days the patient suddenly experienced a serious convulsive hypoglycemic episode accompanied by unconsciousness and a decrease in the blood sugar level to 43 mg per cent. During the next day the patient remained in a serious hypoglycemic condition, blood sugar levels varying between highs of 34 and 61 mg per cent. The hypoglycemia could be combated only by continuous glucose administration (260 gm) part being given intravenously. We also administered injections of Soludacortin and adrenalin. A repeat of the loading test done with 100 gm of dextrose and performed on the third day brought about blood sugar levels of 70 to 88 mg per cent, with one peak of 97 mg per cent.

Because of this markedly greater hypoglycemic action it would be possible

to treat with smaller doses of chlorpropamide diabetics who require far more exogenous insulin, this would not generally be the case with tolbutamide. This action, however, may be regarded as an advantage only if prolonged use and careful laboratory testing show that the chronic toxic effects of the drug or its degradation products have no deleterious effects, particularly on the liver, kidneys, and the hematopoietic systems. The problem of toxicity in this regard is of special interest because we may assume that the ability of chlorpropamide to reduce blood sugar is due to its considerably delayed excretion and that the prolonged effectiveness of the lower dosages is due primarily to cumulative action.

In view of the above evidence and its substantiation by clinical observation, we can state that the initial adjustment period for chlorpropamide is longer (as much as one week) and the duration of action more prolonged than with tolbutamide.

Of 215 patients (including outpatients) untoward side reactions were observed more often at the beginning of the treatment and less frequently after therapy had been in progress for some time. Gastrointestinal disturbances were noted in 10.7 per cent of cases, dermatological side reactions in 3.5 per cent and intolerance to alcohol in 11.6 per cent.

Gastrointestinal disturbances occurred in twenty-three cases and manifested themselves as loss of appetite (often to a great degree), malaise, and occasional nausea and vomiting. Sometimes these disturbances were associated with other complaints in the upper abdomen (for example, pressure on the stomach, gastric cramps and bloating) and, although in only a few cases, with diffuse abdominal colic and constipation. Incidence of these side reactions lowered dramatically when the initial dosage or maintenance dosages were reduced substantially. This, however, necessitated a prolonged initial period of adjustment. Thus the disturbances proved to be dependent on

of the skin was noted in 4. The latter symptom never became so marked that it necessitated discontinuation of the preparation, and it subsided without requiring therapy. In 2 cases of morbilliform exanthemas, we were able to continue medication under antiallergic protection. Marked changes in the differential blood count of the white blood corpuscles (for example, the eosinophils) were not observed in association with the above skin reactions.

As to the hematopoietic system, no toxic effects were noted except for occasional granulocyte decrease which, as proved by periodic follow ups, was only transitory. No hepatic damage or adverse effect on the kidneys or the central nervous system became manifest during the time of observation, which was as long as five months.

Flushing, subjectively often felt as "congestion" and a warmth of the face and neck, visible as a temporary redness sometimes extended to the upper thoracic area. It was observed in 31 patients (regularly in 24) after consumption of even a small quantity of alcohol. The above-mentioned symptoms generally occurred 10 to 15 min. after taking a small quantity of alcohol,

regardless of the type of alcohol imbibed and were similar to an Antabuse alcohol reaction. Of course these side reactions may be prevented by avoidance of alcohol.

A few patients complained of giddiness and buzzing in the ears; these symptoms however cannot be clearly designated as side reactions because their interpretation allows so many other possibilities to be taken into consideration.

#### *Conclusion*

It may be stated that chlorpropamide has two advantages over the other antidiabetic sulfonylurea preparations: first, minimal dosages will produce an entirely satisfactory hypoglycemic effect that compares favorably with that of the other antidiabetic drugs previously used; and second, it is distinguished by an especially prolonged duration of hypoglycemic action, so that in no case need it be given more often than once a day and it may be given as infrequently as every other day. A higher incidence of dyspeptic disturbances must be looked upon as a disadvantage; however, these disturbances were observed principally during initiation of therapy when relatively high doses were being given.



# CHLORPROPAMIDE IN THE MANAGEMENT OF THE ADULT DIABETIC PATIENT\*

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This article will describe our experiences with chlorpropamide in the clinical management of adult diabetic patients

## Methods and Results

Our observations were made on 32 diabetic patients who received chlorpropamide for as long as 36 weeks (FIGURE 1) The study included 20 females and 12 males, with an age range of 33 to 74, 26 were in the range of 50 to 70

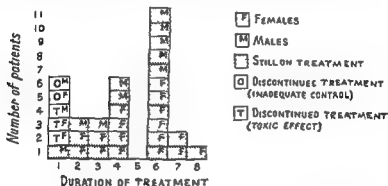


FIGURE 1 Duration of chlorpropamide therapy in 32 patients

(TABLE 1) Constant dietary regimen has been maintained, most of the patients having 2000 cal diets consisting of 250 gm carbohydrate, 90 gm protein, and 70 gm fat—divided into meals of approximately equal caloric value This basic diet plan has been changed in a few cases where body weight indicated need for adjustment Thirteen patients were observed initially in the hospital and 19 have been handled entirely as outpatients Diabetic control has been checked by frequent fasting and 2 hour postbreak fast plasma glucose analyses<sup>1</sup> and frequent determinations of 24 hour glycosuria<sup>2</sup>

A systematic study of possible toxicity has been carried out and has included frequent clinical observation of the patient There have been periodic determinations of body weight serum cholesterol, BSP retention, total and fractional serum proteins, cephalin cholesterol flocculation, serum alkaline phosphatase, icterus index, blood urea nitrogen, urine analysis, and platelet and complete blood counts There have been occasional prothrombin time determinations Serum diastase, transaminase, and bili

\* The work on which this paper is based was supported by a grant from Chas Pfizer & Co, Brooklyn N Y, who supplied the chlorpropamide used in the study

rubin levels have been investigated in a few of the cases. Needle biopsies of the liver also have been obtained in a few cases.

In general, our earlier cases received initial doses of 1.5 to 1.0 gm. The frequency of mild hypoglycemic reactions caused us to decrease the initial dosage to 0.5 gm. or less by the time we had had experience with the first 15 patients (TABLE 2). Only 1 patient received an initial dose of more than 1.0 gm. Chlorpropamide was discontinued in 2 patients because of inadequate control, both had had good control on insulin. In one chlorpropamide was started with progressive decreases in insulin but the improvement was

TABLE 1  
AGE DISTRIBUTION AND STATE OF CONTROL OF 32 PATIENTS WITH DIABETES MELLITUS

Age range	No. of cases	Control on chlorpropamide	
		Adequate	Inadequate
30-40	1	1	0
40-50	4	4	0
50-60	11	10	1
60-70	15	14	1
70-80	1	1	0
Total	32	29	3

transient as demonstrated by increasing glycosuria and insulin requirements. In the other case brought to the hospital in a state of insulin produced hypoglycemia, an attempt made to control the patient with chlorpropamide alone was unsuccessful. Chlorpropamide was discontinued in 3 cases because of possible toxicity. In 2 others, chlorpropamide was discontinued temporarily and resumed without further difficulty when it was decided that accompanying skin reactions were unrelated to the drug.

#### *Clinical Observations*

In assessing control, the same criteria were used as in judging effectiveness of tolbutamide in the therapy of adult diabetes mellitus.<sup>2,4</sup> Adequate control was designated good or fair. A patient was considered to have a good response if one of the following phenomena was observed: a disappearance of the insulin requirement in the insulin-treated patients; a disappearance of glycosuria with an associated decline in plasma glucose levels in the noninsulin-treated glycosuric patients; or a marked fall in the fasting and postprandial plasma glucose levels in the aglycosuric patients when compared to similar levels without chlorpropamide. These biochemical aspects of control were considered together with the following clinical objectives: clinical well-being at all times (that is, freedom from any symptoms); achievement and maintenance of ideal body weight; absence of ketosis and hypoglycemic reactions. A given patient was considered to have a fair

TABLE 2  
SUMMARY OF 32 ADULT DIABETIC PATIENTS TREATED WITH CHLORPROPAMIDE

Case No.	Sex	Age	Duration		Complications	Date of initial treatment	Date treatment discontinued	Initial dose (gm.)	Maintenance dose (gm.)	Response*
			Diabetes (years)	Insulin treatment	Tolbutamide treatment					
1	F	66	3	—	—	11/30/57	—	1.0	0.1	U
2	F	57	0	17 days	—	1/58	—	0.5	0.1	O
3	F	51	10	2 1/2 years	—	1/16/58	—	1.0	0.5	U
4	F	43	0	0	0	1/20/58	—	1.5	0.5	U
5	M	61	20	10 years	2 weeks	2/27/58	3/20/58	1.0	1.0	U
6	M	40	19	—	2 months	1/23/58	—	1.0	0.25	U
7	F	46	1	0	6 months	1/24/58	—	1.0	0.5	U
8	M	66	20	5 years	—	1/28/58	—	1.0	0.5	U
9	M	52	3	3 years	—	2/6/58	—	1.0	0.25	U
10	M	68	18	20 years	0 months	2/6/58	—	1.0	0.5	U
11	M	53	14	—	—	2/9/58	—	1.0	0.25	U
12	F	65	1	1 year	—	2/13/58	—	1.0	0.7	U
13	F	63	20	20 years	—	2/13/58	—	1.0	0.5	U
14	F	45	—	—	—	2/24/58	—	1.0	0.1	U
15	F	62	22	16 years	—	2/20/58	—	0.5	0.5	U
16	F	68	5	5 years	—	4/3/58	—	0.5	0.1	U
17	F	46	—	—	—	4/9/58	7/1/58	0.25	0.1	U
18	F	46	3	—	—	4/10/58	—	0.5	0.1	U
19	M	49	1	3 years	10 months	4/17/58	—	0.5	0.5	U
20	F	74	2	—	—	4/17/58	—	0.5	0.5	U
21	F	59	2	—	—	4/17/58	—	0.1	0.3	U
22	M	48	—	—	—	4/19/58	—	0.25	0.25	U

TABLE 2 (Contd in ref)

Case No	Sex	Age	Duration		Complications	Date of initial treatment	Date treatment discontinued	Initial dose (gm)	Maintenance dose (gm)	Response <sup>a</sup>
			Diabetes (years)	Insulin treatment						
21	F	61			Obesity	4 30 58		0.25	0.2	U
24	M	64	4	—	ISCVD	5 12 58		0.1	0.1	U
25	F	59	8	—	ISCVD	7 3 58	7 17 58	0.25	0.25	U
26	M	64		—	ISCVD coronary disease	5 29 58		0.2	0.2	U
27	F	45		—	ISCVD	6 12 58		0.25	0.5	U
28	F	58	5	1 year		6 17 58		0.5	0.5	U
29	M	59	26 years		(ataxacts osteoarthritis	6 20 58	7 11 58	0.5	0.5	U
30	M	69	10 years			7 4 58		0.5	0.5	U
31	F	75	7 years		(Hypertension osteoarthritis	7 11 58	7 31 58	0.5	0.5	U
32	F	67	22 years		ASCVD neuropathy	7 14 58	7 23 58	0.5	0.5	U

<sup>a</sup> Key: U good I fair O inadequate

TABLE 3  
CONTROL ACHIEVED WITH CHLORPROPAMIDE THERAPY

Patients	Adequate		Inadequate	Total
	Good	Fair	Poor	
Number	22*	8	2	32
Percentage of total	68.7	25.0	6.3	100

\* Includes three patients who showed good results for 2 to 3 weeks but in whom treatment was discontinued because of toxicity

response when the above criteria were partially fulfilled. TABLE 3 summarizes our experience. Thirty of our patients showed adequate control. 22 showed very good response, and 8 showed a fair or partial response. Two patients showed no response; both were diabetics of long standing (more than 20 years), with substantial insulin requirements.

This investigation has little statistical significance. However, observations in a few cases raised interesting questions. Case 11, M P, is a 53 year old male who had had mild diabetes for 14 years and who had had no previous therapy other than avoidance of sweets (FIGURE 2). Outpatient observations had established fasting plasma glucose levels at approximately 275 mg per cent and 2 hour postbreakfast levels of more than 350 mg per cent. His response to 10 gm of chlorpropamide was immediate, the dose was

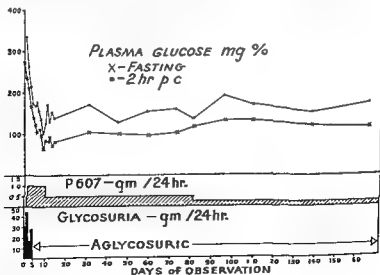


FIGURE 2 Case 11 M P male, 53 years old. Duration of diabetes 14 years. No previous treatment.

reduced to 0.5 gm on the tenth day because the plasma glucose level had fallen to 66 mg per cent fasting and 105 mg per cent 2 hours after breakfast. On this dosage he was observed as an outpatient; plasma glucose levels remained in the range of 100 to 110 mg per cent fasting and 130 to 160 mg per cent 2 hours after breakfast. Further reduction in chlorpropamide to 0.25 gm once daily before breakfast has resulted in slight increase in the

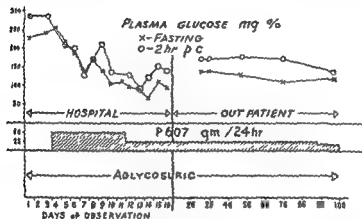


FIGURE 3 Case 18, I R female 46 years old diabetic 3 years. Previous treatment diet.

Case 18, I R a 46 year old female with diabetes of 3 years' duration and treated by diet alone was a glycosuric throughout our observations (FIGURE 3). Her response in the plasma glucose levels to the initial dose of 0.5 gm chlorpropamide was dramatic and compelled reduction to 0.25 gm on the ninth day. There has been progressive weight loss from 173 to 163 lb and the patient is well maintained currently on 0.2 gm chlorpropamide daily.

Case 26, A G a 64 year old male with newly discovered diabetes was handled entirely as an outpatient. His pretreatment fasting and 2 hour

satisfactory response.

The postprandial plasma glucose level as an indicator of therapeutic effectiveness impressed us in evaluating tolbutamide therapy.<sup>2,4</sup> The three cases presented illustrate the same phenomena and the longer term observations support our impression that the prompt and substantial fall in the postprandial plasma glucose level bespeaks a good total response.

The information that approximately five days were required on a given

dosage of chlorpropamide to achieve a blood level plateau, and that the levels fell slowly in the two or three days following cessation of the drug immediately suggested the possibility that we might observe in some of our cases a 'chlorpropamide hang over' effect in the plasma glucose level.<sup>4</sup> We believe we have observed this in one case.

Case 31, S T is a 65 year old white female, diabetic for 7 years who had been managed for the most part with diet alone, but who had also taken as much as 15 U of insulin for short periods (FIGURE 5). Initially observed in the hospital she seemed resistant to 0.5 gm chlorpropamide given as a single daily dose before breakfast. After 6 days of this therapy, the fasting plasma glucose level was 189 mg per cent and the 2 hour postbreakfast level was

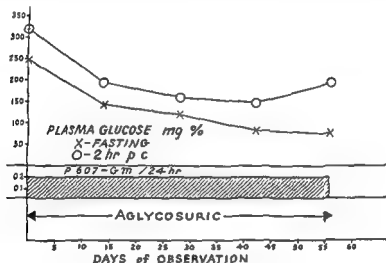


FIGURE 4 Case 26 A G male 64 years old new diabetic

235 mg per cent. She received placebo therapy the next 2 days, on the third day, plasma glucose levels were 145 mg per cent and 246 mg per cent respectively. The relatively lower fasting plasma glucose level and the virtually unchanged 2 hour postprandial level after 2 days of placebo therapy suggested the possibility of a 'hang over' effect. The more favorable response when the drug was resumed remains unexplained. There have been no correlation studies between serum levels of chlorpropamide and plasma glucose levels. Observations in this case have led us to institute such studies. If the observation is validated chlorpropamide may have a significant potential for successful intermittent therapy.

#### Observations Concerning Toxicity

With respect to toxicity, the last case that of S T showed a cephalin cholesterol flocculation after instituting chlorpropamide therapy. transaminase levels.

results. In this case we were fortunate to have needle biopsies of the liver before starting chlorpropamide therapy, and these were repeated as soon as we had confirmed the three-plus cephalin cholesterol flocculation reaction. No evidence of necrosis or any other change was detected with hematoxylin and eosin stain. One other case presented the same cephalin cholesterol flocculation abnormality with no other abnormality in the liver profile. Needle biopsies of the liver were not obtained. In both cases the drug was stopped immediately.

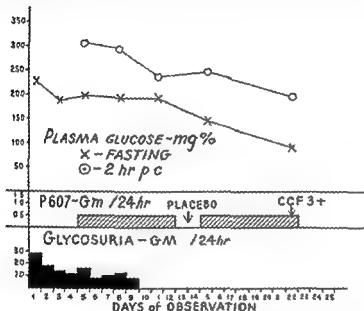


FIGURE 5 Case 31 S T female 65 years old diabetic 12 years

In one other case the drug was stopped because the patient complained of nausea and a constant disagreeable metallic taste. This patient had done well previously on tolbutamide and at his insistence was returned to this therapy.

#### Summary

Thirty two adult diabetic patients receiving chlorpropamide have been observed closely for periods as long as 36 weeks. Thirty of the patients showed adequate response. 22 showed a very good response and 8 a fair response. Two of those showing a good response developed positive cephalin cholesterol flocculation reactions after about 3 weeks of therapy and the drug was discontinued. Two showed no response both of these had had diabetes for more than 20 years and had had substantial insulin requirements. No patients with juvenile type of diabetes were included in these studies.

During observations it was noted in one instance that there was an appar-



ent 'hang-over' effect on blood sugars from the chlorpropamide given forty-eight hours previously. The possibility that intermittent therapy is feasible is suggested and is being studied. Normal needle biopsy liver tissues before therapy and at the time one patient developed a positive cephalin cholesterol flocculation reaction show a dissociation between biopsy results and liver function tests. With available tests, hepatotoxicity is extremely difficult to assess.

### References

1. SOMOGYI M. 1945. A new reagent for the determination of sugars. *J Biol Chem* 160: 61.
2. SOMOGYI M. 1941. A rapid method for the estimation of urine sugar. *J Lab Clin Med* 28: 1220.
3. BRAVERMAN A F \ DREV & S SHERRY. 1956. Experience with Orinase in the management of adult diabetes. *Metabolism* 11 5(6): 911-918.
4. SHERRY S L, ZEFFREN A E, BRAVERMAN A F & W DREV. The use of tolbutamide in the management of adult diabetes. *Ann N Y Acad Sci* 71(1): 249-255.
5. IZZONI D G. Personal communications.

## COMPARATIVE HYPOGLYCEMIC EFFECTS OF CHLORPROPAMIDE, TOLBUTAMIDE AND FURFURYLUREA\*

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This study of the relative potency of the several hypoglycemic agents in altering the blood sugar and serum electrolytes in diabetes mellitus also permits some speculation on the possible sites of action in individual patients

### *Materials and Methods*

Observations were made on adult as well as juvenile diabetic patients. Age at the onset of diabetes and at the time of these studies, the sex of the patient and the dosage and type of insulin taken if any are recorded in TABLE 1

Chlorpropamide, tolbutamide or furfurylurea † 30 or 10 gm. was administered after an overnight fast to 9 juvenile and 17 adult diabetics. The venous blood sugar and the levels of inorganic phosphorus,  $\text{CO}_2$ , potassium, sodium, and chloride in serum from venous blood were measured at hourly intervals for 5 hours in control studies made both prior to and after the ingestion of the hypoglycemic agent. One week or more elapsed between the administration of chlorpropamide, tolbutamide or furfurylurea. All studies were conducted on an outpatient basis after maintaining the subjects on rapid-acting insulin for 2 or more days.

### *Results*

**Control studies** From the control observations (FIGURE 1) it may be seen that in most patients the blood sugar levels remained relatively close to the zero value or declined slightly. In a few patients a distinct spontaneous rise or a definite fall, that is, 40 mg per cent or more was recorded (FIGURE 1). These limits were selected because such changes were rare in the control periods; there were but 2 such patients in each category. These were not confined to either the juvenile or adult group and were unrelated to either the type of insulin employed prior to these studies or to the usual daily dosage of insulin.

**Chlorpropamide effects (30 gm dosage)** Chlorpropamide decreased the fasting blood sugar levels 40 mg per cent or more in 14 of the patients including 2 of the juvenile diabetics. In 2 others, 1 adult and 1 juvenile type diabetic the blood sugar rose by 40 mg per cent or more. Decreases were

\* The study reported in this paper is part of the Pittsburgh Plan for Heart Disease Control and was aided by grants from the John A Hartford Foundation Inc., New York, N.Y., the Public Health Service, Bethesda, Md., and Chas. Pfizer & Co. Inc., Brooklyn, N.Y.

† The structural formula of furfurylurea is 1-( $\beta$ -tolylsulfonyl)-3-furfurylurea. This compound was supplied by The National Drug Company, Philadelphia, Pa.

recorded most consistently in patients in whom the starting blood sugar was between 120 and 260 mg per cent (FIGURE 2)

*Comparisons of chlorpropamide, tolbutamide, and furfurylurea effects at 30 and 10 gm dosages* Seven of the patients given 30 gm of chlorprop-

TABLE 1

Patient	Sex	Age, years	Age at onset of diabetes, years	Usual insulin dosage, U
Juveniles				
W C	M	13	10	25 (NPH)
N G	M	13	12½	3 (NPH)
R L	M	33	10	24 (Protamine Zinc I)
				54 (Regular)
D M	M	14	13	No insulin
E M	F	16	12	46 (Lente)
B M	M	10	7½	30 (Crystalline)
L P	M	16	16	12 (NPH)
C R	F	21	2	65 (Protamine Zinc)
D S	M	16	13	40 (Protamine Zinc)
				60 (Regular)
Adults				
G C	F	32	25	18 (NPH)
W E	M	32	20	50 (NPH)
J E	F	24	1	42 (Protamine Zinc)
				22 (Regular)
A H	F	60	60	No insulin
P K	F	76	70	15 (Protamine Zinc)
A L	F	43	27	44 (NPH)
A M	F	63	63	No insulin
M M	F	49	40	25 (Lente)
B P	F	56	46	30 (NPH)
J S	F	56	56	No insulin
R N	F	54	46	No insulin
H F	F	55	47	No insulin
L V	F	58	48	No insulin
C S	F	56	55	No insulin
J A	F	56	56	No insulin
L R	M	61	57	No insulin
A B	F	56	49	No insulin

amide were subsequently tested with similar amounts of tolbutamide and of furfurylurea, and then with 10 gm of chlorpropamide and of furfurylurea. The blood sugar changes obtained are shown in FIGURE 3. TABLE 2 is based on—greater or less than minus 40 mg per cent together

side or  
agents,



furfurylurea lowered the blood sugar 40 mg per cent or more in 3 of 5 patients with levels of 120 mg per cent or less at zero time

Finally reducing the dosage of chlorpropamide from 3 gm to 1 gm produced a decrease of blood sugar of 40 mg per cent or more in only 1 of the

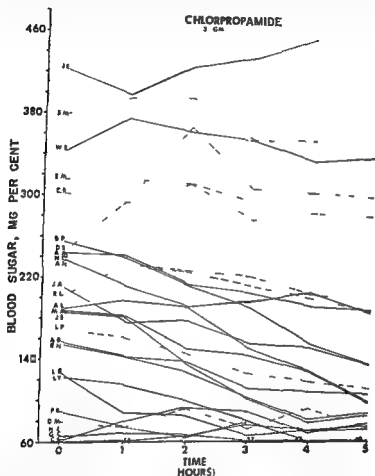
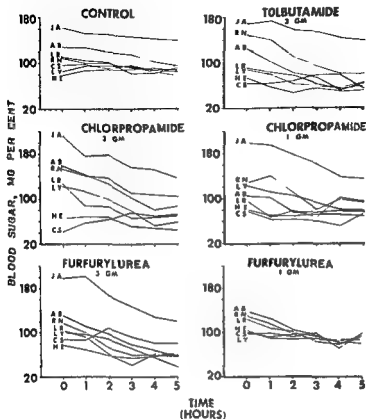


FIGURE 2 Hypoglycemic effects of chlorpropamide The administration of 3.0 gm of chlorpropamide at minus 45 min produced decreases in blood sugar of 40 mg per cent or more in 12 of the patients including 2 juvenile diabetics

patients In 1 other RN a change of this magnitude was partially cancelled by a subsequent rise This contrasts with the 3.0 gm studies in which all of the patients for whom such a comparison is available developed a distinct relative or absolute hypoglycemia Furfurylurea reduced to the dosage of 1.0 gm, decreased the responses to none of 6 in contrast to 5 of 7 with 3.0 gm

*Serum inorganic phosphorus changes in relation to blood sugar alterations*  
In FIGURE 4 the changes in blood sugar at 1, 2, 3, 4 and 5 hours in relation to



\* starting blood sugar less than 120 mg per cent

the fasting levels have been plotted against the increment or decrement in blood sugar. If decrease of

drops in blood sugar in still other patients the blood sugar actually increased

### Discussion

Although the data presented indicate that not all patients are equally responsive to chlorpropamide or other oral insulin substitutes, decreases in

blood sugar can occur with chlorpropamide in both juvenile and adult type diabetes. Insofar as relative efficacy in this regard is concerned chlorpropamide and furfurylurea appeared to be more effective than tolbutamide in this small group of patients. It is noteworthy, too, that none of the other agents tested produced responses in patients unaffected by chlorpropamide (TABLE 2). Finally, although chlorpropamide and tolbutamide each appeared to be more effective when the zero value lay between 120 and 260 mg per cent furfurylurea alone acted at levels below 120 mg per cent.

It is well established that serum inorganic phosphorus decreases quite regularly with the acceleration of carbohydrate metabolism in nondiabetic

TABLE 2  
HYPOGLYCEMIC RESPONSES IN THE SAME ADULT DIABETIC PATIENTS TO  
VARIOUS ORAL INSULIN SUBSTITUTES

Patient	Control	Chlorprop- amide 30 gm	Chlorprop- amide 10 gm	Tolbut- amide 30 gm	Furfuryl- urea 30 gm	Furfuryl- urea 10 gm
J A	-	+	+	-	+	
A B	-	+	-*	+	+	-
R N	-*	+	-	+	+	-
L R	-*	+	-*	-*	+	-
L Y	-*	+	+	-*	+	-*
H E	*	-*	-*	-*	-*	-*
C S	*	-*	-*	-*	-*	-*
Blood sugar more than -40 mg per cent	0.7	5.7	2.7	2.7	5.7	0.6

\* Zero blood sugar less than 120 mg per cent

patients by glucose insulin or glucose and insulin in combination and upon insulin injection in those diabetic patients previously shown to be unable to dispose of glucose loads at a normal rate and to develop a significant drop in inorganic phosphorus.<sup>1</sup> This decrease in serum inorganic phosphorus can be taken to reflect disposal of glucose by anaerobic glycolysis and formation of high energy phosphorous compounds, since deposition of liver glycogen does not segregate phosphate.<sup>2,3</sup>

It may be assumed that diabetic patients maintained on diet alone possessed some ability to elaborate insulin. It would then appear, from those cases in which no significant decrease was observed in the serum inorganic phosphorus concomitant with the decrease in blood sugar, that chlorpropamide and the other agents tested had not increased anaerobic glycolysis (FIGURE 4). The observed decreases in blood sugar without concomitant hypophosphatemia are to be attributed therefore to decreased glycogenolysis and gluconeogenesis or increased deposition of glucose as liver glycogen, or both, a process that, unlike anaerobic glycolysis does not pre-empt inorganic phosphorus from the serum. In patients in whom such hypoglycemic changes occurred with a concomitant drop in inorganic phosphorus, anaerobic glycolysis

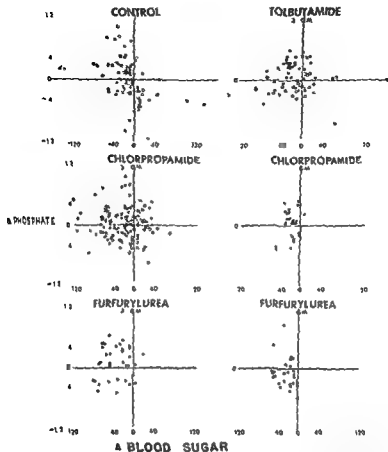


FIGURE 4 Correlation of serum inorganic phosphorus and blood sugar changes in 15 identical adult subjects on insulin with and without various drugs.

It is apparent that on many occasions the blood sugar decreased without concomitant significant fall in serum inorganic phosphorus. These decreases may be

due to a direct effect of the drug on the insulin action or from accentuation or prolongation of the effect of such exogenous insulin.

presumably was accelerated either by discharging insulin, by accentuating its action or survival or by increasing the permeability of cells to glucose.

These comparisons are not offered, of course with any assurance that the drugs tested would necessarily prove effective in reducing the insulin requirement or in regulating the diabetes in these patients. Also, it is possible that



in keeping with the experience of others with agents other than chlorpropamide, some of the patients who did not show a positive response to a particular drug initially might do so after several weeks of treatment.<sup>6</sup>

### *Summary and Conclusions*

It is possible that responses to single dosages of oral hypoglycemic agents may differ from those obtained after repeated or prolonged therapy. The following statements therefore apply only to the single dosage experiments herein described.

Certain diabetics did not respond to single dosage of chlorpropamide tolbutamide or furfurylurea with a decrease in blood sugar.

Chlorpropamide administered in 3 gm doses at minus 45 min produced during the next 5 hours a decrease of 40 mg per cent or more in the zero blood sugar level of most of the adult diabetics and in 2 of 7 juvenile diabetics or patients whose diabetes began during childhood. During control observations comparable decreases occurred only occasionally.

Chlorpropamide and furfurylurea tested in equal dosages in the same patients produced greater response than did tolbutamide.

Decreases with any of the agents tested were noted most consistently in patients in whom the zero blood sugar level lay between approximately 120 and 260 mg per cent.

Furfurylurea may differ from the other agents in possessing a greater ability to lower blood sugar levels when the starting values are less than 120 mg per cent.

The absence of significant decreases in the serum inorganic phosphorus in patients in whom the blood sugar fell significantly would suggest that in general the hypoglycemic responses in this group of diabetics are attributable to decreased gluconeogenesis and glycogenolysis or to segregation of glucose in the liver as glycogen—a process that unlike anaerobic glycolysis does not lower serum inorganic phosphorus. In those in whom both the blood sugar and the serum inorganic phosphorus fell, an increase in anaerobic glycolysis can be postulated—this as a result of a greater output survival or effectiveness of endogenous insulin or as a consequence of an increase in the permeability of cells to glucose.

### *References*

1. DANOWSKI, T. B. 1957. Diabetes Mellitus with Emphasis on Children and Young Adults. Chaps. 9 and 10. Williams & Wilkins Co. Baltimore, Md.
2. FENN, W. O. 1939. The deposition of potassium and phosphate with glycogen in rat livers. *J. Biol. Chem.* **128**: 297.
3. FENN, W. O. & L. F. HAEGE. 1940. The deposition of glycogen with water in the livers of cats. *J. Biol. Chem.* **136**: 87.
4. VILLEE, C. A., H. W. DEANE & A. B. HASTINGS. 1949. Synthesis of glycogen *in vitro* by rat diaphragm muscle. *J. Cellular Physiol.* **34**: 159.
5. NICHOLS, N. 1955. The effect of glycogen deposition on liver phosphorus. *J. Clin. Invest.* **34**: 1710.
6. PENNOCK, L. L. 1957. Sulfonylureas in the treatment of diabetes. *Penn. Med. J.* **60**: 613.

# CHLORPROPAMIDE IN SINGLE DOSAGE AND THE RESPONSES TO INSULIN OR GLUCOSE IN DIABETICS THE ABSENCE OF EVIDENCE OF INCREASED GLUCOSE UTILIZATION\*

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In responsive patients with diabetes mellitus, chlorpropamide in single dosage lowers blood sugar levels, often without a concomitant decrease in serum inorganic phosphorus<sup>1</sup>. This suggests that, in patients who develop relative or absolute hypoglycemia after a single dose without significant

TABLE I

Patient	Sex	Age, years	Ages at onset of diabetes years	Usual insulin dosage, U
Juveniles				
N G	M	13	12½	3 (NPH)
R L	M	33	10	24 (Protamine Zinc I)
			14	54 (Regular)
D M	M	14	13	No insulin
F M	F	16	12	46 (Lente)
S M	M	10	7½	30 (Crystalline)
L P	M	16	16	12 (NPH)
C R	F	21	2	65 (Protamine Zinc)
D S	M	16	13	40 (Protamine Zinc)
				60 (Regular)
Adults				
G C	F	32	25	18 (NPH)
W E	M	32	20	50 (NPH)
A L	F	43	27	44 (NPH)
M M	F	49	40	25 (Lente)
B P	F	56	46	30 (NPH)
J McH	M	37	21	111 (Regular)
J S	F	56	56	No insulin

hypophosphatemia, the chief or, perhaps sole action of this agent is segregation of glucose as glycogen (a process not associated with deposition of phosphate) or suppression of gluconeogenesis or glycogenolysis. To examine further the possibility that there is a simultaneous increase in glucose utilization, glucose tolerance tests and insulin tolerance tests with and without

\*The study reported in this paper is part of the Pittsburgh Plan for Heart Disease Control, and was aided by grants from the John A Hartford Foundation Inc., New York, N Y, the Public Health Service, Bethesda Md and Chas Pfizer & Co.,<sup>2</sup> Brooklyn N Y

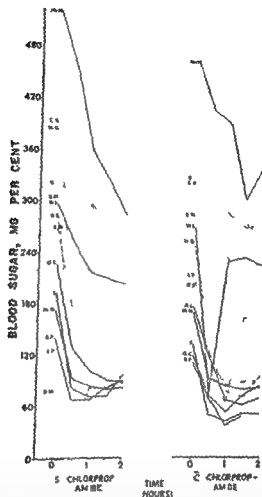


FIGURE 1 Blood sugar changes during insulin tolerance test in diabetics with and without 3.0 gm of chlorpropamide at minus 45 min. Comparison of the changes at 0  $\frac{1}{2}$ , 1  $\frac{1}{2}$ , and 2 hours in the 2 groups shows no accentuation of hypoglycemia after chlorpropamide (TABLE 2). Broken lines identify juvenile diabetics.

chlorpropamide administration in single dosage were undertaken, and the changes in serum inorganic phosphorus and other electrolytes were measured.

#### Materials and Methods

The clinical histories of the diabetic patients studied (some with juvenile diabetes mellitus and others with diabetes that began during the adult years) are summarized in TABLE 1. In most of the glucose tolerance tests 0.5 gm of glucose per kilogram of body weight was administered intravenously at zero time as a 50 per cent solution in water. In a few patients 1.75 gm of glucose per kilogram of body weight was used orally. In the

insulin tolerance tests 0.1 U of commercial rapid acting crystalline insulin (per kilogram of body weight), diluted 1:9 with normal saline for ease of measurement, was injected intravenously at zero time. Chlorpropamide (30 gm) was then given 45 min before the start of the second glucose or insulin tolerance test. Blood sugar and serum electrolytes were measured just prior to and at intervals after the administration of the sugar or the insulin.

TABLE 2  
STATISTICAL EVALUATION OF BLOOD SUGAR AND SERUM INORGANIC PHOSPHORUS CHANGE DURING INSULIN TOLERANCE TESTS IN DIABETIC SUBJECTS\*

Period	Hours	No. of patients	Mean	±S.D.
Blood sugar				
Control	1 $\frac{1}{2}$	12	-84.9*	35.9
Chlorpropamide	1 $\frac{1}{2}$	12	-5	39.8
Control	1	12	110.8	43.6
Chlorpropamide	1	12	-87.8	42.6
Control	1 $\frac{1}{2}$	12	-119.8	46.0
Chlorpropamide	1 $\frac{1}{2}$	12	-82.7	41.9
Control	2	11	-126.8	51.8
Chlorpropamide	2	11	-89.8	42.6
Serum inorganic phosphorus				
Control	1 $\frac{1}{2}$	10	-0.590	0.445
Chlorpropamide	1 $\frac{1}{2}$	10	-0.451	0.256
Control	1	10	-0.649	0.399
Chlorpropamide	1	10	-0.440	0.391
Control	1 $\frac{1}{2}$	11	-0.509	0.423
Chlorpropamide	1 $\frac{1}{2}$	11	-0.397	0.434
Control	2	-	-0.570	0.322
Chlorpropamide	2	-	-0.294	0.416

\* Difference is statistically significant.

### Results

**Insulin tolerance tests.** TABLE 1 summarizes the blood sugar changes in the insulin tolerance tests with and without the prior administration of 30 gm chlorpropamide at minus 45 min. Patients whose diabetes began during childhood are indicated by broken lines. The solid lines identify those who were usually required inulin in the dosage,

adult diabetics. In fact, the blood sugar decrease at one half hour was somewhat less after chlorpropamide (TABLE 2). It is probable that the week-to-week fluctuations in the starting blood sugar levels obscured the decrement often produced in fasting levels by chlorpropamide. Nevertheless, the fact

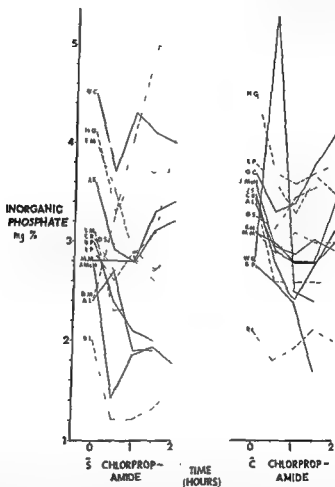


FIGURE 2 Serum inorganic phosphorus changes during insulin tolerance test in diabetics with and without 30 gm of chlorpropamide. As in the blood sugar changes there is no evidence that the serum inorganic phosphorus responses are regularly affected by chlorpropamide (TABLE 2).

that insulin preceded by chlorpropamide failed to produce a decrement in blood sugar greater than that seen with insulin alone argues against an augmentation of insulin action by this oral insulin substitute when it is given in single dosage. It also eliminates the possibility that a single dosage of chlorpropamide enhanced endogenous insulin output in these particular

patients, it provides no information of course on the effects of repeated doses or prolonged therapy.

Chlorpropamide did not increase the magnitude of the hypophosphatemia produced by insulin alone (TABLE 2, PART 2). Since increased utilization of glucose by means of anaerobic glycolysis increases the drop in serum inorganic phosphorus, the apparent absence of such a change in these experiments can be cited as further evidence against a release of endogenous insulin.

TABLE 3  
STATISTICAL EVALUATION OF BLOOD SUGAR AND SERUM INORGANIC PHOSPHORUS CHANGES DURING IV GLUCOSE TOLERANCE TESTS IN DIABETIC SUBJECTS

Period	Hours	No. of patients	Mean	S.D.
Blood sugar				
Control	1/2	8	124	8.4
Chlorpropamide	1/2	8	88.1	1.4
Control	1	8	104.0	21.4
Chlorpropamide	1	8	68.6	60.3
Control	1 1/2	8	71.2	58.8
Chlorpropamide	1 1/2	8	68.2	68.2
Control	2	8	82.1	39.0
Chlorpropamide	2	8	38	60
Serum inorganic phosphorus				
Control	1/2	8	0.228	0.206
Chlorpropamide	1/2	8	0.064*	0.190
Control	1	8	0.280	0.251
Chlorpropamide	1	8	-0.011*	0.158
Control	1 1/2	8	-0.262	0.263
Chlorpropamide	1 1/2	8	-0.184	0.220
Control	2	8	-0.122	0.224
Chlorpropamide	2	8	0.146*	0.281

\* Differences are statistically significant.

or prolongation or potentiation of the action of exogenous insulin by chlorpropamide in single dosage.

*Intra-venous glucose tolerance tests.* The administration of chlorpropamide 45 min. before the intravenous glucose produced a decrease in the increment in the blood sugar at the half hour point (TABLE 3).

The administration of chlorpropamide decreased the usual hypophosphatemic response to glucose loads. The comparisons of the actual changes in blood sugar and serum inorganic phosphorus recorded during the glucose tolerance tests are summarized in TABLE 3. The differences in the blood sugar at the half hour, and in the serum inorganic phosphorus at the half, one-, and two hour points are statistically significant.

It would appear that under the conditions of these experiments chlorpropamide did reduce the blood sugar response at the half hour point, but it

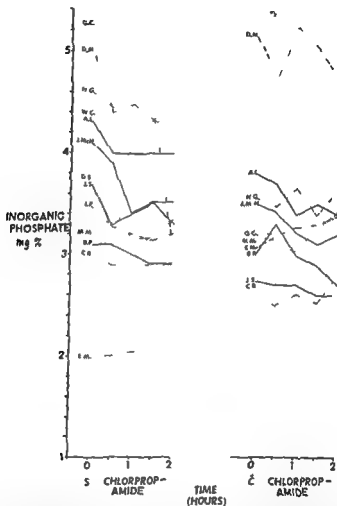


FIGURE 3 Blood sugar changes during glucose tolerance tests in diabetics with and without 3.0 gm of chlorpropamide at minus 45 min. Solid lines identify adult diabetics and broken lines juvenile diabetics who received 0.5 gm of glucose intravenously at zero time. Dotted lines represent patients who received 1.75 gm of glucose per kilogram of body weight by mouth at zero time. In the intravenous glucose tolerance tests chlorpropamide decreased the degree to which the blood sugar rose at the half hour point (TABLE 3).

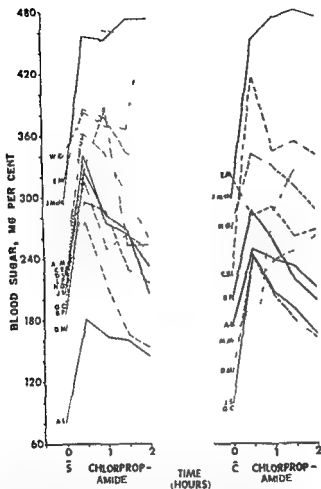


FIGURE 4 Serum inorganic phosphorus changes during glucose tolerance test with and without 30 gm of chlorpropamide. Symbols are as defined in FIGURE 3. The serum inorganic phosphorus response to intravenous glucose was less after chlorpropamide than the 1 $\frac{1}{2}$ , 1, and 2 hour points in TABLE 3.

the serum total  $\text{CO}_2$  content.<sup>2</sup> In diabetics not receiving insulin the administration of glucose intravenously tends to raise the serum potassium and lower the serum total  $\text{CO}_2$  content.<sup>2</sup> The  $\text{pH}$  responses were not altered by chlorpropamide administration.

#### Discussion

In these studies in keeping with findings on analysis of tissues<sup>2,6</sup> it has been assumed that deposition of liver glycogen preempts potassium but not



inorganic phosphorus. The concomitants of muscle and other tissue glycogen formation are not known. If it is true that glycogen deposition does not segregate inorganic phosphorus, then the decrease in serum inorganic phosphorus reflects anaerobic glycolysis in cells and indicates either the formation of glucose phosphates or high energy phosphorus compounds or some other metabolic process that pre-empt's this ion.

In the studies herein described there was no evidence that the hypophosphatemic action of insulin or of glucose was enhanced by chlorpropamide in single dosage. This finding argues against either a direct action of chlorpropamide in single dosage on anaerobic glycolysis or an effect upon exogenous or endogenous insulin. The latter point is perhaps debatable since no direct information is available on the endogenous supplies of this hormone in our patients. However it should be pointed out that in those of our subjects whose diabetes was controlled without insulin or with insulin in tiny amounts a significant residual capacity to produce insulin can be postulated. However even these failed to show a greater drop and therefore no evidence can be brought forth in support of chlorpropamide action on the beta cells or on insulin secretion when the drug was administered in single dosage.

Published studies designed to clarify the mechanism or mechanisms of action of the earlier oral hypoglycemic agents have at times led to diametrically opposed conclusions. These and other discrepancies are undoubtedly attributable to variables such as species differences, dosage and schedule variations and the characteristics of individual diabetics. Confining ourselves to measuring the responses to a single and often large dosage of an oral hypoglycemic agent of the sulfonylurea type administered orally 45 min before the test procedure at intervals of 1 week or more, we have found that the hypoglycemic responses observed under these circumstances are frequently unaccompanied by a decrease in serum inorganic phosphorus. This argues against a significant component of insulin discharge, insulin potentiation, insulin survival or direct action on cells in those instances of hypoglycemia since certainly at least the first three of these present in sufficient degree would be expected to lower the serum inorganic phosphorus. Although the indirect nature of the evidence offered must be admitted, the possibility is raised that the oral hypoglycemia agents of the sulfonylurea type elicit at least a 2 phase response. Initially they act to suppress glycogenolysis and gluconeogenesis and segregate glucose as glycogen. Endogenous insulin released in amounts too small to affect serum inorganic phosphorus could play a role in this, if such an action of insulin is accepted. With continued therapy the beta cells are stimulated to elaborate insulin in quantities sufficient to produce the expected changes in serum inorganic phosphorus. Such a sequence of events would account for some of the discrepancies in published reports of the effects or lack of effects of sulfonylureas on serum inorganic phosphorus or on other evidences of endogenous insulin production.

#### Summary and Conclusions

In view of the fact that deposition of hepatic glycogen does not segregate phosphate in the liver, the decrease in serum inorganic phosphorus which in

nondiabetics, invariably accompanies acceleration of carbohydrate metabolism by exogenous insulin or by glucose loads, can be taken as an index of enhanced disposal of glucose by anaerobic glycolysis.

The failure to observe any increment in the hypophosphatemia characteristic of insulin or of glucose tolerance tests in these particular diabetic patients following chlorpropamide suggests that chlorpropamide in single dosage at least, did not accelerate anaerobic glycolysis either directly, by evoking endogenous insulin secretion, or by accentuating the action or survival of either exogenous or endogenous insulin. Of course, this does not exclude such a response in other diabetics nor the possibility that repeated or prolonged therapy might provide evidence of an effect of chlorpropamide on anaerobic glycolysis unrelated or related to insulin.

### References

1. DINOWSKI T S & J M MATTER. 1959. Comparative hypoglycemic effects of chlorpropamide, tolbutamide and furfurylurea. *Ann N Y Acad Sci* 74 3: 971.
2. DINOWSKI T S. 1957. Diabetes Mellitus with emphasis on Children and Young Adults. Chaps 9 & 10. Williams & Wilkins Co. Baltimore Md.
3. FENN W O. 1939. The deposition of potassium and phosphate with glycogen in rat livers. *J Biol Chem* 128: 297.
4. FENN W O & I F HAEGE. 1940. The disposition of glycogen with water in the livers of cats. *J Biol Chem* 138: 87.
5. VILLEY C A, H W DEANE & A B HASTINGS. 1949. Synthesis of glycogen *in vitro* by rat diaphragm muscle. *J Cellular Physiol* 34: 159.
6. NICHOLS N. 1955. The effect of glycogen deposition on liver phosphorus. *J Clin Invest* 34: 1710.

# CORTISONE ENHANCEMENT OF PERIPHERAL UTILIZATION OF GLUCOSE AND THE EFFECTS OF CHLORPROPAMIDE\*

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Available evidence indicates that the hyperglycemia associated with excesses of ACTH and adrenocortical type steroids, which is largely attributable to increased gluconeogenesis, may be based in part on a blockade of insulin action or glucose utilization in the tissue.<sup>1,2</sup> An extensive series of

TABLE I  
FASTING HYPOPHOSPHATEMIA PRODUCED BY THE ADMINISTRATION OF CORTISONE 45 MINUTES BEFORE TEST

Sex	Cortisone mg	Blood sugar mg %			Serum inorganic phosphorus <sup>a</sup> , mg %		
		Mean	+S D	No. tested	Mean	±S D	No. tested
Healthy adults (21 to 60 years)							
M	0	84.3	11.1	64	3.43	0.34	67
M	200	81.5	11.3	60	3.23	0.56	61
F	0	80.7	11.6	71	3.58	0.55	70
F	200	81.9	12.4	64	3.37	0.30	65
Relatives of juvenile diabetics (21 to 60 years)							
M	0	91.5	13.2	67	3.32	0.59	60
M	200	87.9	10.9	26	2.97	0.49	26
F	0	85.0	11.6	69	3.41	0.56	67
F	200	84.5	9.6	21	3.12	0.46	27

\* Differences between the bracketed groups are statistically significant

glucose tolerance tests (with serum inorganic phosphorus measurements) conducted in this laboratory on relatives of juvenile diabetics, on apparently healthy persons, on healthy dental students, and on adult diabetics suggests that under the conditions of our experiments, cortisone probably enhances the net utilization of glucose by anaerobic glycolysis as a result of increased gluconeogenesis by stimulation or enhancement of insulin action or by facilitating the entry of glucose (alone or in combination) into cells.

\* The study reported in this paper is part of the Pittsburgh Plan for Heart Disease Control and was aided by grants from the John A Hartford Foundation Inc., New York, N.Y., the Public Health Service, Bethesda, Md., and Chas Pfizer & Co. Inc., Brooklyn, N.Y.



*Materials and Methods*

Adult relatives of juvenile diabetics free of clinical symptoms of diabetes mellitus, apparently healthy adult male and female university personnel, healthy male dental students, and adult diabetics who did not require insulin received 1.75 gm of glucose per kilogram of body weight with or without the administration, 45 min earlier, of 200 mg of cortisone by mouth. Blood

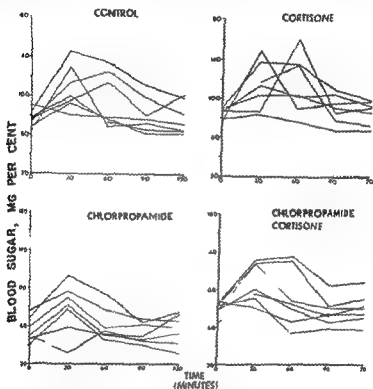


FIGURE 1 Blood sugar changes in healthy dental students during oral glucose tolerance test. Glucose was administered at 1.75 gm/kg of body weight with and without cortisone or chlorpropamide alone or in combination.

for sugar and serum inorganic phosphorus analysis was withdrawn just prior to the administration of the glucose and at intervals thereafter, and was analyzed by methods in regular use in this laboratory.<sup>8</sup> The studies with cortisone and chlorpropamide, alone or in combination, were limited to the dental students and to the adult diabetics.

*Results and Comments*

*Fasting blood sugar and glucose tolerance tests with and without cortisone in healthy adults with and without diabetic offspring.* TABLE I is made up of the mean fasting blood sugar and serum inorganic phosphorus values in healthy

university personnel and in relatives of juvenile diabetics in the 21 to 60 year age range with and without the prior administration of 200 mg of cortisone 45 min earlier. In addition to lower fasting blood sugar values in the healthy university personnel both male and female (indicated by the single asterisk) it is noteworthy that the administration of cortisone invariably produced lower levels of serum inorganic phosphorus (measured 45 min

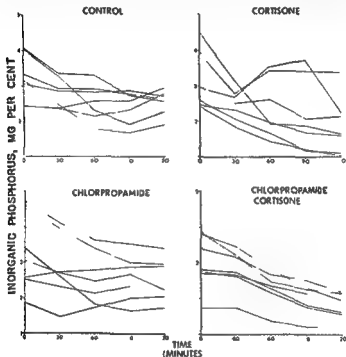


FIGURE 2 Serum inorganic phosphorus changes in healthy dental students during oral glucose tolerance test. Glucose was administered at 1.75 gm/kg of body weight with and without cortisone or chlorpropamide alone or in combination.

adults is in all probability an enhancement rather than a blockade of glucose utilization

The magnitude of the decline and the results of urinary analyses in selected patients indicated that the bulk of the observed decrease in serum inorganic phosphorus cannot be attributed to losses in urine

TABLE 2 shows the levels of blood sugar and serum inorganic phosphorus found one half hour and 2 hours following the administration *per os* of 1.75 gm of glucose per kilogram of body weight at zero time with and without 200 mg of cortisone given 45 min earlier. In no instance did the steroid

TABLE 3

BLOOD SUGAR AND SERUM INORGANIC PHOSPHORUS CHANGES\* DURING INTRAVENOUS GLUCOSE TOLERANCE TEST FOLLOWING ADMINISTRATION OF CORTISONE, CHLORPROPAMIDE OR BOTH

	Cortisone	Chlorpropamide	Cortisone and chlorpropamide
Blood sugar	No change	Lower initial and final sugars with usual rise Blood sugar less than 60 mg per cent reached in 4 of 7 patients (Decreased gluconeogenesis or increased hepatic glycogenation without increased extrahepatic utilization)	No change
Serum inorganic phosphorus decrement	Greater (Increased gluconeogenesis enhanced insulin or both)	No change	Greater (In nondiabetic controls chlorpropamide did not alter cortisone effects)

\* Compared to controls

affect the levels of blood sugar nor did it alter the serum inorganic phosphorus levels present at the 30 min point. However, in each instance these amounts of cortisone greatly enhanced the usual hypophosphatemia usually doubling the magnitude of the decrease

glycemic response obtained with 1.75 gm of glucose per kilogram of body weight given at zero time. On the other hand the prior administration of

it is evident  
that students  
the hyper

moreover, that hypoglycemia, that is, values below 60 mg per cent developed in 4 of 7 subjects. Finally, the combination of cortisone and chlorpropamide in the dosages and at the time cited (FIGURE 1) canceled the general lowering of the blood sugar curves noted with chlorpropamide alone restoring them

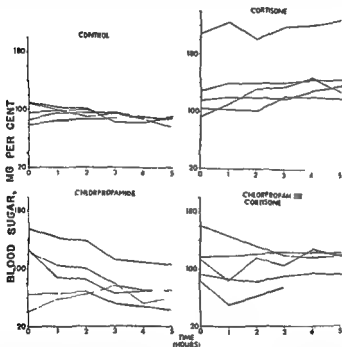


FIGURE 3 Blood sugar changes during oral glucose tolerance test. Glucose administered 175 gm/kg of body weight in diabetics with and without cortisone or chlorpropamide alone or in combination.

to the levels seen in the control studies or in those made with cortisone alone. Certainly the absolute hypoglycemia seen with chlorpropamide alone did not develop.

From FIGURE 2 it is evident that cortisone administration at minus 45 min increased the hypophosphatemia that is a regular feature of such intravenous glucose tolerance tests in nondiabetic subjects. In the studies with chlorpropamide alone (FIGURE 2), in the majority of which the blood sugar reached absolute hypoglycemic levels, the serum inorganic phosphorus did not decrease beyond the range seen in the control observations and certainly did not fall as low as in the cortisone experiments. In the combination of



cortisone and chlorpropamide (FIGURE 2) the serum inorganic phosphorus changes resembled those seen with cortisone. The changes in the blood sugar and the serum inorganic phosphorus under these four circumstances are summarized in TABLE 3.

The greater degree of hypophosphatemia in the cortisone studies suggests that this steroid increased the utilization of glucose by anaerobic glycolysis, by discharging insulin, prolonging or potentiating insulin action or by

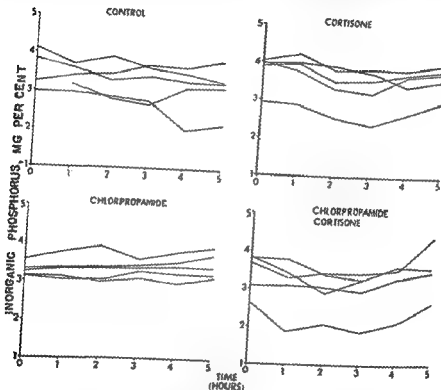


FIGURE 4. Serum inorganic phosphorus changes during oral glucose tolerance test. Glucose was administered at 1.75 gm/kg of body weight in diabetics with and without cortisone or chlorpropamide alone or in combination.

increasing cell permeability. Increased gluconeogenesis could have occurred and potentiated this hypophosphatemic response by providing an increased glucose load.

The lower blood sugar levels at zero time, throughout the course of the glucose tolerance tests and at their end, suggest that chlorpropamide decreased gluconeogenesis or enhanced the supplies or action of insulin or both. However, the fact that the serum inorganic phosphorus changes were no greater than those seen in the control studies argues for the former as the predominant or sole action.

*Cortisone and chlorpropamide effects in diabetics* FIGURES 3 and 4 show the blood sugar and serum inorganic phosphorus levels in diabetics during several samplings for five hours with or without the prior administration of cortisone or chlorpropamide alone or in combination.

In contrast to the shorter studies in the healthy university personnel in the relatives of diabetics and in the healthy dental students the blood sugar levels tended to rise when cortisone was given alone (FIGURE 3). However the same trend to hypophosphatemia noted in the three other groups was observed (FIGURE 4). Chlorpropamide alone tended to lower the blood sugar without decrease in the inorganic phosphorus (FIGURES 3 and 4).

In the chlorpropamide plus-cortisone studies it appears that chlorpropamide tended to produce an initial lowering of the blood sugar in three of the subjects (FIGURE 3); this was maintained in only one. The serum inorganic phosphorus changes (FIGURE 4) assumed the pattern seen with cortisone alone.

Under the conditions of these experiments, chlorpropamide partially modified the hyperglycemia of cortisone administration, probably by suppressing gluconeogenesis. Since the serum inorganic phosphorus changes of the cortisone experiments were not attenuated, it may be argued that chlorpropamide did not exert any significant action on any endogenous insulin that these patients may have had. Alternatively, it is possible that a slight enhancement was evoked just sufficient to offset the decrease resulting from the lesser degree of gluconeogenesis.

#### Summary and Conclusions

*Fasting blood sugar and glucose tolerance tests with and without cortisone in healthy adults and in relatives of juvenile diabetics* The administration of 200 mg of cortisone 45 min. before an oral glucose tolerance test (1.75 gm/kg of body weight) lowered the fasting and the 2 hour levels of serum inorganic phosphorus without discernible change in the control blood sugar concentrations or increments. This suggests that under the conditions of these experiments, in nondiabetic subjects, this steroid enhanced the utilization of glucose by anaerobic glycolysis as reflected by significant increases in the usual hypophosphatemia. This could represent increased gluconeogenesis, increased secretion, intensification or prolongation of insulin action, or increased cell permeability to glucose alone or in combination. These findings argue strongly against a peripheral blockade of insulin action in these experiments.

*Action of chlorpropamide upon cortisone hypophosphatemia* Chlorpropamide administered to healthy nondiabetic male dental students 45 min. before an intravenous glucose tolerance test did not modify either the usual increment in blood sugar or the usual decrement in serum inorganic phosphorus, although the absolute levels of blood sugar at the start, during and at the end of the tolerance test were significantly lowered and hypoglycemia appeared in the majority.

The failure to observe a further decrease in the serum inorganic phosphorus during these glucose tolerance tests suggests that the chief mode of action of the chlorpropamide was decreased gluconeogenesis or increased removal of extracellular glucose to form hepatic glycogen. This is concluded since

both of these processes are known to lower the blood sugar without segregation of serum inorganic phosphorus.

Our observations suggest that in the quantities and under the conditions employed cortisone cancels the hypoglycemic effect of chlorpropamide presumably by overriding the inhibition of gluconeogenesis by the latter but that chlorpropamide does not interfere with the cortisone enhanced hypophosphatemia.

Blood sugar and serum inorganic phosphorus changes indicate that cortisone when first given to healthy subjects enhances rather than decreases the net disposal of glucose by processes that preempt serum inorganic phosphorus.

*Cortisone and chlorpropamide effects in diabetics.* In adult diabetics not requiring insulin 200 mg. of cortisone given *per os* at minus 45 min. tended to raise the venous blood sugar levels during several samplings for 5 hours while chlorpropamide had the opposite effect. When the two were administered together the blood sugar levels dropped initially in 3 of 5 subjects, but the decrease was maintained in only 1 of these. These findings suggest that, in the amounts given, chlorpropamide could not completely overcome the hyperglycemic action of cortisone.

Insofar as the serum inorganic phosphorus levels are concerned, no changes were observed with chlorpropamide while with cortisone or with cortisone and chlorpropamide a tendency to decrease was noted. This again indicates that under these experimental conditions chlorpropamide did not cancel the action of cortisone.

### References

1. BASTENIE P. A., J. R. M. FRANCELSON & V. CONARD. 1953. Effect of cortisone on carbohydrate metabolism measured by the glucose assimilation coefficient. *J. Clin. Endocrinol. Metabolism* 13: 865.
2. BASTENIE P. A., V. CONARD & J. R. M. FRANCELSON. 1954. Effect of cortisone on carbohydrate metabolism measured by the glucose assimilation coefficient. *Diabetes* 3: 200.
3. BORNSTEIN J. & C. K. PARK. 1953. Inhibition of glucose uptake by the serum of diabetic rats. *J. Biol. Chem.* 205: 503.
4. STADIE W. C., V. HALGAARD & J. B. MARCH. 1952. The effect of growth hormone and cortisone on the action of bound insulin. *J. Biol. Chem.* 198: 785.
5. KERPOLA W. 1953. Accumulation of pyruvic acid in the blood during treatment with cortisone or ACTH and in Cushing's syndrome. *Acta med. Scand.* 145: 357.
6. FRANKLYN T. F., J. J. GARRETT & P. J. ROSEN. 1954. Metabolic adjustments during stress: the effect of intravenous glucose on pyruvate during compound F infusion. *J. Clin. Endocrinol. Metabolism* 14: 791.
7. THURBERG T. 1953. Occurrence and significance of citric acid in the animal organism. *Physiol. Revs.* 33: 1.
8. INGLES D. J. 1950. Biologic properties of cortisone: review. *J. Clin. Endocrinol.* 10: 1317.
9. DANCOWSKI T. S., L. GEFENMAN, F. M. MATEER, W. B. PARSONS, F. A. WEIGAND, H. VERMELSTEIN, J. H. PETERS. 1951. Carboxylic cation exchange resin effects in dogs. *J. Clin. Invest.* 30: 984.

# INFLUENCE OF CHLORPROPAMIDE UPON POSTPANCREATECTOMY AND SIMULTANEOUS DIABETES\*

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Despite the current uncertainty regarding the mechanism of the hypoglycemic effect of the aryl sulfonylureas the observation of their failure to lower the blood sugar in severe alloxan diabetes in animals<sup>1</sup> and in depancreatized animals<sup>2</sup> and man<sup>3,4</sup> remains unchallenged. Although negative results were anticipated because of the structural similarity of chlorpropamide to carbutamide it was considered important to observe the action of the newer agent in pancreatectomized patients.

Two patients in whom complete pancreatectomy had been performed by Porter and his associates<sup>5</sup> were selected. One was a 60 year old woman 8 years after the operation the other a 55 year old man 16 months after removal of the pancreas. In both surgery had been performed for pancreatic carcinoma. Neither had evidence of recurrent or persistent tumor at the time of these studies.

Analyses tests of liver function (cephalin flocculation thymol turbidity serum bilirubin serum alkaline phosphatase serum cholesterol and serum protein electrophoresis) and measurements of phenolsulfonphthalein excretion and blood urea nitrogen. Blood sugar levels were determined by the method of Folin and Wu at frequent intervals. Those obtained 3 hours after breakfast are shown in FIGURES 1 to 3. Urinary glucose was estimated 5 times daily by the qualitative Benedict's method the mean daily value is shown in the figures. Serum chlorpropamide (CPA) concentrations were determined by the ultraviolet spectrophotometric method developed by Toolan and Wagner.<sup>6</sup>

Following the initial period chlorpropamide was administered in a dose of 1 gm. daily and the insulin dose was simultaneously reduced from 20 to 10 U. of NPH in the first patient M. B. (FIGURE 1). During the next 3 days glycosuria increased markedly and the patient developed thirst and polyuria. The dose of chlorpropamide was raised to 2 gm. on the fourth day after which it was discontinued owing to the appearance of nausea and vomiting. The serum chlorpropamide level at this time was 30 mg. 100 ml. The first day

Satis

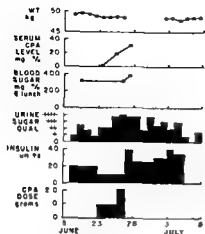
week

lorprop

amide daily after the control period. The previous dosage of 40 U. of NPH

\* The work reported in this paper was supported in part by a grant from Chas. Pfizer & Co. Inc. Brooklyn N. Y.

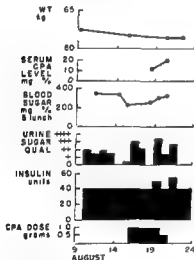
M B 60 Q # 259 PANCREATCTO 11 1952



	CONTROL	R <sub>1</sub>	DAYS of R <sub>1</sub>
HGB	12.8	11.3	4
WBC	8,100	8,100	4
R	54	59	4
L	44	39	4
M	2	2	4
URINE			
SG	1,010		
ALBUMIN	1+		
BUN	23	28	4
CEPH FLOCC	0	0	4
THYMOL TURBID	0	0	4
BLIRUBIN	TRACE	TRACE	4
ALK P TASE	12.7 KAU	16 KAU	4
CHOLESTEROL			
TOTAL	209	167	4
ESTERS	151	106	
PROTH TIME	14 sec	13	4
R <sub>1</sub> TRACER	22%	16%	4

FIGURE 1 Response to chlorpropamide of patient M B six years after pancreatectomy

R R 55 R # 314651 PANCREA ECTOMY 1957



	CONTROL	R <sub>1</sub>	DAYS of R <sub>1</sub>
HGB	12.0		
WBC	13,150	14,840	5
P	40	30	5
L	57	61	5
E	2	3	5
B	1	6	5
PLTS	385,000	459,000	4
URINE			
SG	1,004	1,020	5
ALBUMIN	0	±	5
GLUCOSE	0	4+	5
BUN	22		
PSP	75%		
ALK P TASE	12.7 KAU	15.8 KAU	5

FIGURE 2 Response to chlorpropamide of patient R R sixteen months after pancreatectomy

insulin was continued because of persistently elevated blood sugar levels. Despite the addition of chlorpropamide, with serum concentrations to 20 mg/100 ml, the blood sugar rose and glycosuria increased during the experimental period.

Laboratory studies (FIGURES 1 and 2) indicated the presence of a mild neutropenia of questionable significance, which increased in R R after chlorpropamide administration. Minimal albuminuria also was observed in this patient after treatment with this agent. The fall in serum cholesterol

in M. B. is difficult to evaluate because of major alterations in food intake during part of the period. The serum alkaline phosphatase concentrations increased in the 2 patients from 12.1 to 16 and from 12.7 to 15 King Armstrong units (normal, 12 or less), respectively.

Chlorpropamide was also given to 11 patients with spontaneous, stable diabetes that began after 30 years of age. Among them were 5 women and 1 man ranging in age from 58 to 81 years and, in insulin requirement, from 20 to 60 U. Insulin was discontinued and 0.5 to 1 gm daily doses of chlorpropamide were given for periods of 2 weeks to 4 months. In 4 members of

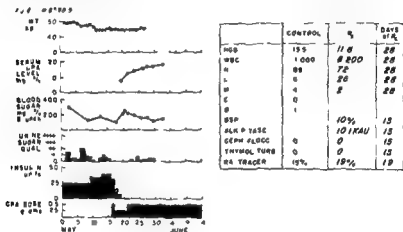


FIGURE 3 Response to chlorpropamide of a patient with spontaneous diabetes

this group, blood sugar concentrations were maintained at 200 mg/100 ml or less 3 hours after breakfast. FIGURE 3 summarizes the observations in a patient whose blood sugar levels were satisfactorily controlled by chlorpropamide alone. This agent replaced 30 U of glotins insulin in an obese 77 year-old woman following an unsuccessful trial of tolbutamide.

Of the 2 patients who failed to respond to tolbutamide,

one was substituted successfully for 40 U of protamine zinc insulin. The other, a 68 year old man well regulated on 20 U of protamine zinc insulin, had also failed to respond to tolbutamide. Although an initial course of chlorpropamide restored the blood sugar values to essentially normal levels, a second trial of this agent after 1 week on tolbutamide was ineffective in preventing hyperglycemia.

The other 7 patients who responded to chlorpropamide

that in neither was control of the blood sugar adequate prior to chlorprop

amide administration. Parallel with previous observations on other aryl sulfonylureas Root has reported the potentiation of insulin action by chlorpropamide in 2 totally depancreatized dogs. Such an effect was not found in the present study on 2 human subjects, although it should be noted that the agent was given for much shorter periods. In one of Root's dogs the serum alkaline phosphatase increased and a fatty liver was found at autopsy. The significance of the small increases in alkaline phosphatase levels in the 2 patients reported here requires further evaluation. In the 6 patients with spontaneous diabetes no disturbance of liver or kidney function was seen during chlorpropamide treatment nor was there evidence of alteration of the bone marrow or of allergic reaction.

*Effect of Chlorpropamide upon Plasma Unesterified Fatty Acid Concentrations*

The importance of plasma unesterified fatty acids (UFA or NEFA) in lipid transport and their relationship to carbohydrate metabolism have been

TABLE 1  
PLASMA UNESTERIFIED FATTY ACID LEVELS FOLLOWING ORAL CHLORPROPAMIDE\*

Time (min)	Serum chlorpropamide				Blood sugar				Unesterified fatty acids			
	Patient				Patient				Patient			
	a†	b	c	d	a	b	c	d	a	b	c	d
	(mg/100 ml)				(mg/100 ml)				(ml g/l)			
0	0	0	0	0	132	110	84	217	0.50	0.36	0.33	0.67
60				2.9	132	114	82	196	0.34	0.24	0.36	0.47
165	24				112	84	61	164	0.27	0.16	0.31	0.43
225		25	20		108	93	63	115	0.34	0.35	0.36	0.24
300		24	25	18		92	59	70		0.48	0.43	0.50

\* Patients a, b, and c were given 3 gm of chlorpropamide; patient d received 2 gm.

† Patient a took NPH insulin 10 U daily until 48 hours prior to this experiment; patients b, c, and d required no insulin.

demonstrated by Dole,<sup>8</sup> Gordon,<sup>9</sup> and others. Elevated levels in diabetic patients in the postabsorptive state and during ketosis have been demonstrated by Bierman.<sup>10</sup> The high UFA concentrations found in acidosis fall sharply in response to insulin. The hypoglycemic effect of tolbutamide is accompanied by a decline in plasma UFA concentrations.<sup>11</sup>

In the present study, 4 patients with mild diabetes were given a single 2 or 3 gm oral dose of chlorpropamide after a 9 or 14 hour period of fasting. Only 1 member of the group required insulin; this was discontinued 48 hours prior to the study. Plasma levels of sugar, chlorpropamide, and UFA were

etermined at interval for 3 hours The method of Corliss as used for  
 UFA estimation

The results are listed in table 1 The blood sugar decreased in all patients  
 as the serum chlorpropamide level rose to 18 mg/100 ml or more The  
 UFA concentration fell in 3 patients as unchanged and at the end of  
 the study rose again

# References

Chlorpropamide like tolbutamide and tolbutamide an effective oral  
 hypoglycemic agent in some patients with diabetes Failure of chlorpropa-  
 midamide to produce a decrease in the blood sugar level in the absence of the  
 pancreas has been observed in 10 patients A small decrease in serum alkaline  
 phosphatase level was found in both pancreatotomy and pancreatoma after treat-  
 ment with chlorpropamide for blood sugar control Plasma unsaturated fatty ac-  
 id levels decreased after chlorpropamide as they do after tolbutamide in a similar

# References

We are indebted for technical assistance to Barbara Jones and Emily B. Jones  
 and also to Margaret Hawthorne of the Metabolism Ward of the University  
 of Illinois at Chicago

# References

1. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
 sugar level in patients with diabetes mellitus. *Ann. N.Y. Acad. Sci.* 10: 408-410  
 2. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
 sugar level in patients with diabetes mellitus. *Ann. N.Y. Acad. Sci.* 10: 408-410  
 3. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
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 4. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
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# References

1. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
 sugar level in patients with diabetes mellitus. *Ann. N.Y. Acad. Sci.* 10: 408-410
2. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
 sugar level in patients with diabetes mellitus. *Ann. N.Y. Acad. Sci.* 10: 408-410
3. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
 sugar level in patients with diabetes mellitus. *Ann. N.Y. Acad. Sci.* 10: 408-410
4. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
 sugar level in patients with diabetes mellitus. *Ann. N.Y. Acad. Sci.* 10: 408-410
5. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
 sugar level in patients with diabetes mellitus. *Ann. N.Y. Acad. Sci.* 10: 408-410
6. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
 sugar level in patients with diabetes mellitus. *Ann. N.Y. Acad. Sci.* 10: 408-410
7. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
 sugar level in patients with diabetes mellitus. *Ann. N.Y. Acad. Sci.* 10: 408-410
8. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
 sugar level in patients with diabetes mellitus. *Ann. N.Y. Acad. Sci.* 10: 408-410
9. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
 sugar level in patients with diabetes mellitus. *Ann. N.Y. Acad. Sci.* 10: 408-410
10. Jones, B. A. and Jones, E. M. 1959 The effect of tolbutamide on the blood  
 sugar level in patients with diabetes mellitus. *Ann. N.Y. Acad. Sci.* 10: 408-410



11. BIERMAN, I. I., T. A. ROBERTS & V. P. DOLE. 1957. Effect of tolbutamide on plasma non esterified fatty acids. *Proc Soc Exptl Biol Med* 95: 437.
12. GORDON, R. S., JR. 1957. Unesterified fatty acid in human blood plasma. II. The transport function of unesterified fatty acids. *J Clin Invest* 36: 810.

*Discussion of the Paper*

HENRY RICKETTS (*Department of Medicine, University of Chicago School of Medicine, Chicago, Ill.*) I think it is still quite mysterious that the human being without a pancreas and maintained on insulin fails to show a depression of blood sugar when sulfonylureas are added to the regimen in contrast to the totally depancreatized dog in which (as shown by a number of workers including ourselves) the addition of sulfonylureas to suboptimal doses of insulin results in a clear cut depression of blood and urinary glucose. I have yet to hear why the human being responds differently from the dog in this respect, and I wish we could have the answer.

When speaking of the effects of sulfonylureas in depancreatized people or animals we should be careful about our use of the term "potentiation of insulin." Insulin potentiation implies accentuation of the effects of insulin; this is not quite true in depancreatized dogs because as we have demonstrated in a work thus far unpublished in the totally depancreatized dog maintained on suboptimal amounts of insulin and receiving chronically added sulfonylureas there is a clear reduction in blood and urinary sugar with no change of nitrogen excretion. Since the addition of 2 U. of insulin to the regimen in these animals produces the expected decline in blood and urinary sugar and now a clear diminution of nitrogen excretion I do not think we can refer to the sulfonylurea effect as insulin potentiation. I suspect that it is not and I suspect also if our evidence is valid, that one must conclude that in the dog without a pancreas the sulfonylurea effect occurs in other tissues for whose integrity a small minimum of insulin is necessary.

## THE EFFECTS OF CHLORPROPAMIDE ON ENDOCRINE FUNCTION IN PATIENTS WITH DIABETES MELLITUS AND ITS EFFECTS IN OTHER ENDOCRINE DISORDERS\*

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The continued widespread use of tolbutamide for the treatment of diabetes mellitus has left little doubt about either the efficacy of action or the acceptance by both the physician and patient of this oral agent for the treatment of this disease. Similar drugs are destined to undergo investigation, and certain of these will find a place as competitors with, or substitutes for, tolbutamide.

In order to compete with tolbutamide a compound must be at least as potent a hypoglycemic agent and equally innocuous from the standpoint of toxicity. Additional attributes of such a drug might include equal effectiveness at lower dose ranges or blood levels, less expense to the patient, or usefulness as alternate therapy in patients known to be hypersensitive or unresponsive to tolbutamide.

It has been the aim of our study to accumulate data on chlorpropamide regarding each of these points. Preliminary results indicate that chlorpropamide is effective as an oral hypoglycemic agent in the same type of diabetic patient that responds to tolbutamide and that dosages of approximately one third the magnitude of the latter drug result in equal or higher serum levels. Additional data suggest that hypoglycemic reactions are more common with chlorpropamide, but in short term usage few manifestations of toxicity to vital organs were observed. As with other sulfonylurea derivatives slight inhibition of thyroid function may occur but this does not appear to be clinically significant.

### *Methods and Results*

Fifty-four patients were selected for a trial of chlorpropamide therapy because as a group they characterized the diabetic most likely to respond to a sulfonylurea hypoglycemic agent as regards age, sex, body type, duration of diabetes and daily insulin need. The group included 13 recently discovered diabetics who could not be controlled with diet, 13 individuals previously controlled with insulin and 28 patients who had been treated with tolbutamide (TABLES 1, 2, and 3).

Chlorpropamide was administered orally in doses ranging from 0.2 to 1.0 gm daily, and frequently a priming dose of 1 or 2 gm was given. Blood sugar levels were obtained daily in both the fasting and postprandial states.

\* Work reported in this article was supported in part by Research Grant 2A 5118 (C) and (CS) from The National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md., by the Conely Coleman Fund of the Ohio State University, Columbus, Ohio, and by Chas. Pfizer & Co., Brooklyn, N. Y.

TABLE 1  
METHOD OF STUDY OF HYPOGLYCEMIC EFFECT OF CHLORPROPAMIDE

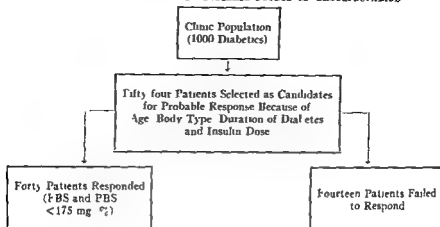


TABLE 2  
CHARACTERISTICS OF RESPONSIVE AND NONRESPONSIVE PATIENTS

Factors	Responsive (40 patients)	Nonresponsive (14 patients)
Mean age (years)	60	56
Percentage female	80	71
Percentage more than 20 lb overweight	75	64
Duration of diabetes (years)	5.6	6.4
Percentage with history of ketosis	3	29
Mean daily insulin (units)	11	25

TABLE 3  
TREATMENT BEFORE CHLORPROPAMIDE

	Responsive (40 patients)	Nonresponsive (14 patients)
Controlled on tolbutamide	13	0
Uncontrolled on tolbutamide	10	5
Uncontrolled on diet alone	10	3
Controlled with insulin	7	6

in hospitalized patients and weekly or biweekly in outpatients. Patients were counted as responsive if all blood sugar levels fell below 175 mg per cent and remained below this level. Chlorpropamide treatment was continued for at least 7 days in nonresponsive patients and usually until the serum level of the drug approached or exceeded 20 mg per cent. Treatment was continued in all patients who responded except in instances where untoward reactions developed.

Forty of the 54 patients met the criteria for response and 14 failed to do so. As a group those responding were slightly older, more obese and had a

TABLE 4  
RESULTS OF CHLORPROPAMIDE TREATMENT IN PATIENTS PREVIOUSLY TREATED WITH TOLBUTAMIDE

Patients	Tolbutamide period		Chlorpropamide period		
	Mean FBS (mg %)	Daily dose (gm)	Mean FBS (mg %)	Daily dose (gm)	1200 level (mg %)
<b>Group 1</b>					
Response to both agents 13 patients	135 (110-164)	1.54 (1.2)	110 (60-162)	0.45 0.2-1.0	11 (1-20)
<b>Group 2</b>					
Response to chlorpropamide only 10 patients	250 (200-380)	1.60 1.4	121 (80-160)	0.57 (0.2-1.0)	18 (17-20)
<b>Group 3</b>					
Unresponsive 5 patients	241 (215-270)	1.50 (1.2)	229 (215-270)	0.45 (0.2-0.9)	22 (16-29)

shorter duration of the diabetic state than those who did not respond. These differences between the groups were slight and statistically insignificant; however, the mean pretreatment daily insulin dose was 11 U in the responsive group and 25 U in the nonresponsive group, and only 1 of the 40 patients in the former group gave a history of past ketoacidosis while 4 of 14 in the latter group had such a history.

Twenty-eight patients who had taken tolbutamide were subjected to a trial of chlorpropamide in order that blood sugar responses and magnitudes of dosage of the 2 drugs might be compared (TABLE 4). Chlorpropamide in daily doses of 0.2 to 1.0 gm was substituted for tolbutamide in 13 patients (Group 1) who previously had responded in an optimal fashion to the latter drug. The degree of control with chlorpropamide in a mean dose of 0.45 gm daily compared favorably to that obtained with the daily administration of 1.54 gm of tolbutamide, and no patient who previously had been controlled

with tolbutamide was found to be resistant to chlorpropamide. Serum levels of chlorpropamide averaged 13 mg per cent in this group.

Fifteen additional patients had been taking 1.0 to 3.0 gm of tolbutamide daily, but displayed fasting blood sugar levels of 200 to 380 mg per cent and could be classified as relatively resistant to tolbutamide at this dosage level. When chlorpropamide was substituted, 10 of these individuals (Group 2) showed a fall in mean fasting blood sugar levels from 250 to 123 mg per cent and each met our criteria for response. At the time of the favorable response the dose of chlorpropamide ranged from 0.2 to 1.0 gm daily, and serum levels of the drug averaged 18 mg per cent. However, in 5 subjects (Group 3) the fasting blood sugar levels fell only slightly, from a mean of 243 to 229 mg

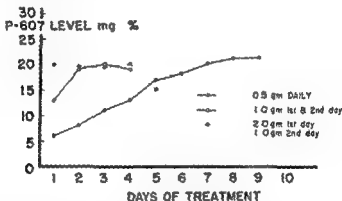


FIGURE 1 Effect of initial chlorpropamide dosage on chlorpropamide blood levels. Solid curve with closed circles represents data from 4 patients treated with dosages shown; the solid curve with open circles 2 patients; and the broken curve 2 patients.

per cent as chlorpropamide in daily doses of 0.25 to 0.50 gm was substituted for an average dose of 1.5 gm of tolbutamide. Serum levels of chlorpropamide ranged from 16 to 29 mg per cent in these unresponsive patients.

It became apparent early in the study that some correlation existed between magnitude of dosage, serum levels of the drug, and response. Accordingly, initial daily doses ranging from 0.5 to 2.0 gm were administered to small groups of subjects, and the relationship between the serum levels obtained and the lapse of time needed for attainment of satisfactory blood sugar levels was noted (FIGURE 1). When a dosage schedule of 0.5 gm daily was employed, about 4 days were necessary to achieve those serum concentrations of the drug that were found to be optimally effective. This dose resulted in progressive elevation of serum levels for 9 to 10 days, after which constant levels were maintained. When larger initial doses were used, higher serum levels were achieved much more rapidly. With either method, optimal control of hyperglycemia was attained only after 4 to 12 days (average 6 days).

Blood levels of chlorpropamide obtained at random in a group of 20 patients maintained for 1 to 20 weeks on a daily dose of 0.5 gm were found to range from 16 to 20 mg per cent (FIGURE 2). In patients in whom the drug was

discontinued, serum levels as high as 5 mg per cent persisted as long as 72 hours. The effect of chlorpropamide upon thyroid function was assayed by determination of serum protein bound iodine (PBI) levels,

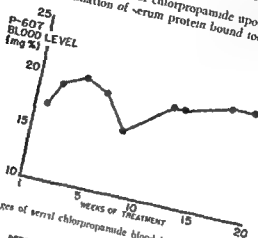


FIGURE 2 Averages of serum chlorpropamide blood levels of 20 patients maintained on 0.5 gm daily

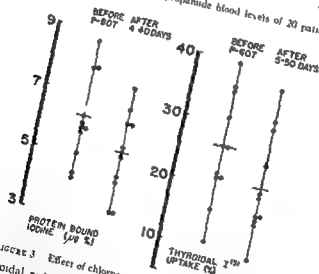


FIGURE 3 Effect of chlorpropamide on thyroid function tests

24 hour thyroidal radioiodine uptakes, and serum cholesterol values, both during the pretreatment period and after 4 to 50 days of therapy and, subsequently, at random intervals. Comparison of these values suggests some inhibition of thyroid function, as the mean PBI fell from 6.2 to a level of 5.4 μg per cent, and the mean uptake fell from 27 to a value of 22 per cent (FIGURE 3). This effect of a sulfonurea derivative has been described by

an improvement in diabetic control rather than an alteration in thyroid function although a direct effect on lipid metabolism cannot be excluded. In 2 patients with concomitant thyrotoxicosis and hyperglycemia, chlorpropamide proved to be an effective hypoglycemic agent.

The relationship of chlorpropamide therapy to adrenal cortical function was studied by quantitative measurement of daily 24 hour urinary excretion

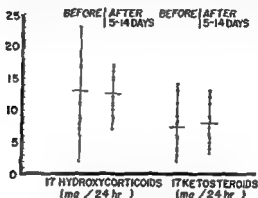


FIGURE 4 Effect of chlorpropamide on urinary steroid excretion

of 17 hydroxycorticoids and 17 ketosteroids. Values for each patient were averaged individually and control levels were compared with those obtained during treatment. No changes attributable to therapy were noted. Corticoids averaged 13.5 mg per day before therapy and 13.0 mg per day during therapy; ketosteroids averaged 8.0 mg per day in the control period and 8.5 mg per day during treatment (FIGURE 4). Each of 3 subjects with Cushing's syndrome due respectively to adrenal cortical hyperplasia, adrenal cortical adenoma, and exogenous administration of 6 methyl prednisolone displayed significant lowering of blood sugar and became normoglycemic with chlorpropamide treatment. This response differs from that reported by others who failed to observe such an effect when using tolbutamide.\*

A 23 year old juvenile diabetic who was also treated had undergone a surgical hypophysectomy in the hope that his severe progressive retinitis proliferans might be controlled. Hypophysectomy resulted in a decrease of his daily insulin requirement from 80 to 14 U of NPH. The daily admin

*Reactions to chlorpropamide.* Numerous tests were made to determine the influence of chlorpropamide on the functions of the liver, kidney, and bone marrow (TABLE 5). Such determinations were made prior to the inception of chlorpropamide therapy and were repeated after 5 to 10 days and later.

at random intervals throughout the period of study. Averages and ranges of values obtained before therapy were compared to the mean determinations made during treatment.

No significant alterations in liver function were evident from the comparison of values of thymol turbidity, serum alkaline phosphatase, serum

TABLE 5  
TESTS OF LIVER, KIDNEY AND BONE MARROW FUNCTION

Test	No. of patients	Values before chlorpropamide	Values after chlorpropamide
Thymol turbidity (units)	16	9 (0-20)	9.4 (0-40)
Serum alkaline phosphatase (SJR units)	14	6.8 (4-14)	6.3 (4-16)
Serum bilirubin (direct and indirect mg %)	14	0.1-0.3 (0.1-0.1-0.0-0.2)	0.2-0.1 (0.1-0.1-0.5-0.4)
Prothrombin concentration (%)	9	83 (52-100)	81 (42-100)
Albumin globulin (gm %)	7	4.3-3.3 (2.6-1-1.8-4.4)	4.1-3.5 (2.5-2.6-4.6-4.0)
Bromsulphalein retention (percentage after 45 min)	10	3.5 (0-14)	2.5 (0-8)
Cephalin flocculation (units)	10	trace (0-2+)	trace (0-1+)
Serum glutamic oxaloacetic transaminase (units)	10	19.7 (11-2)	29.8 (7-103)
Serum creatinine (mg %)	7	1.4 (1.0-1.7)	1.4 (1.0-1.9)
Blood urea nitrogen (mg %)	14	16 (8-31)	18 (8-32)
Serum uric acid (mg %)	9	3.6 (1.0-4.4)	3 (1.0-4.7)
White blood cell count (per mm <sup>3</sup> )	25	7400 (4000-20000)	6400 (3500-10400)
Neutrophils (percentage)	24	61 (43-7)	60 (35-74)
Hemoglobin (gm %)	21	13.9 (12-16)	13.4 (11-16)

bilirubin, prothrombin concentration, serum albumin and globulin, Bromsul-

phalein retention, serum uric acid, white blood cell count, neutrophils, hemoglobin.

4 categories: Of the 54 patients to whom the drug was given, 9 (17 per cent) manifested undesirable effects. Three individuals, all of whom were lean mild diabetics, experienced classic hypoglycemic responses. These episodes



occurred in both the late postprandial and fasting states and were rapidly controlled by glucose administration. Decrease in the maintenance dose abolished these reactions. Two subjects complained of burning midepigastria distress following ingestion of the tablets. Each had registered similar complaints before being placed on therapy but felt that the drug exaggerated the discomfort. This complaint was easily controlled by administering antacids with the drug or by giving the drug with meals. Many patients with varying degrees of severe cerebral arteriosclerosis were treated with chlorpropamide. In 2 of these cerebral vascular accidents were observed during treatment. In another episode of accentuated mental confusion occurred. In none was actual hypoglycemia documented although this would appear to be the most likely explanation for their symptoms.

For the most part no alteration of hepatic function could be attributed to the drug. However in one patient with biopsy proved hepatic cirrhosis slight enlargement of the liver and increase in thymol turbidity titers occurred during chlorpropamide therapy. Similar episodes had occurred during periods when the drug was not being given. There were no manifestations of dermal hypersensitivity to the drug.

#### *Summary and Conclusions*

Chlorpropamide was given to 54 selected diabetic patients and an adequate response was obtained in 40 of these. As a group the patients tended to be mild diabetics requiring little or no insulin, were obese and middle aged or older.

Ten of 15 patients unresponsive to tolbutamide responded optimally to chlorpropamide. No patient responsive to tolbutamide failed to respond to chlorpropamide.

The effective maintenance dose ranged from 0.2 to 1.0 gm. daily and resulted in serum levels of 13 to 20 mg. per cent. A priming dose raised the serum levels more rapidly but failed to hasten the hypoglycemic response.

Slight depression of thyroid function as determined by the usual tests was observed but no clinical manifestations of thyroid deficiency or enlargement of the thyroid gland occurred.

No alterations of adrenal cortical, renal, hematopoietic or cardiac function were observed. Liver function remained normal except in 1 patient with known hepatic cirrhosis.

23 of 54 patients (43 per cent) Of these  
which were  
arteriosclerosis

deteriorated during therapy.

Two patients with thyrotoxicosis and 3 with Cushing's syndrome associated with hyperglycemia became normoglycemic with chlorpropamide treatment.

*References*

- 1 McGAWACK T H 1956 *Metabolism* 5 916
- 2 FAJANS S S L H FOLIN H S SELTZER R D JOHNSON R D GOTTIER A R  
HENNES H I WACHENBERG I P WEERMAN J W COHN 1956 *Metabolism*  
5 820

*Discussion of the Paper*

CARLOS P LAMAR (*Utami Ila*) I propose to make a short comment on another aspect of the side effects of sulfonylureas that I do not believe has been mentioned in these pages.

Hamwi and others reported on the number of patients who showing late developing lack of response or resistance to sulfonylureas were necessarily returned to insulin therapy. We have 7 patients who were originally selected as ideally suited for this type of therapy and who apparently responded well for periods of 2 to 7 months after which it was necessary to return them to insulin. The original daily doses of insulin required for maintenance of adequate diabetic control for periods of several years had ranged between 20 and 30 U. Following the use of tolbutamide and carbutamide in this group, the amount of insulin required to obtain adequate diabetic control ranged from 60 to 90 U, or 3 times the pretolbutamide or carbutamide dose level. These individuals have been observed now for periods of 6 months to almost 2 years and although their insulin requirements are declining gradually, they still require 100 per cent more insulin than they did before.

With respect to Hamwi's cases of cerebral arteriosclerosis with manifestations of clinical hypoglycemia in which he was unable to substantiate the presence of chemical hypoglycemia I suggest a very simple therapeutic test that we have used very successfully for quite some time: administer sugar intravenously even if the individual has a blood sugar level of 250 to 300 mg per cent or more. These figures do not mean that that individual who probably has a decreased transfer or osmosis through the cerebral cell membrane is not hypoglycemic: the proof of hypoglycemia lies in the fact that the administration of glucose under these circumstances is followed by a dramatic correction of the clinical hypoglycemic symptoms. An adjustment in the treatment of these individuals to a less rigid adherence to the concept of so called normal glycaemic levels would help them and perhaps might be a factor in the prevention of those cerebral vascular accidents mentioned as of obscure origin in this and other individuals.

## CLINICAL EXPERIENCE IN THE USE OF CHLORPROPAMIDE IN THE TREATMENT OF DIABETES MELLITUS\*

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*Metabolic Endocrine Division National Institute of Nutrition Mexico D F Mexico*

It is our purpose in this paper to describe our clinical experience with chlorpropamide in the management of the adult and juvenile diabetic patient.

In fifty unselected subjects from the Diabetic Clinic of the Nutritional Disease Hospital in Mexico D F, Mexico, the influence of chlorpropamide has been studied with particular reference to changes in hyperglycemia and the general health of the patient. In a few cases, studies of thyroid function were carried out.

### *Controls*

The hypoglycemic effect of the compound was first tested in 10 healthy normal young adults who were used as a control group. These patients were given glucose tolerance tests using a standard 100 gm oral dose, and measuring blood sugar every hour for 5 hours. This study was repeated on the same subjects 3 to 6 days later, each patient receiving a test dose of chlorpropamide (1 gm) 1 hour before the glucose ingestion.

**Results** The results of this study are shown in FIGURE 1. There is a definite flattening of the curve after chlorpropamide. It is interesting to note the significant depression in the fasting blood sugar values during the test in 6 of the 10 subjects.

### *Diabetic Patients*

**Material and methods** In this group of 50 cases, diabetes was of the adult or stable type in 45 and juvenile or brittle in 5. The duration of the disease ranged from 3 months to 19 years, with an average of 6½ years.

Most patients were between 40 and 50 years old, the ages ranging from 16 to 70 years. The age distribution is shown in TABLE 1. Forty four cases had vascular manifestations of diabetes, among the 50 cases there were 30 other complications as shown in TABLE 2. Before the beginning of the study, 11 patients were on diet alone, 33 were receiving insulin, 5 were on carbutamide or tolbutamide, and 4 were receiving no treatment (TABLE 3).

All patients were kept on a constant calculated diet during a control period and throughout the study. The diet components were measured in terms of familiar household serving practices (ambulatory cases), or were supplied by the dietetic kitchen (hospitalized cases).

Chlorpropamide therapy was usually started in divided doses of 750 mg to 1 gm in 24 hours. The duration of treatment was 8 to 69 days.

Patients who had failed to respond to diet alone or who were on other oral hypoglycemic agents received chlorpropamide as the only medication. The dose of the drug was modified subsequently in each case, according to

\* The work reported in this paper was aided by a grant from Pfizer de Mexico, Mexico D F, Mexico, who supplied the chlorpropamide used.

the initial response. Chlorpropamide was given alone when daily insulin requirements were 20 U or less.

A reduction of 75 per cent of the insulin dose was made in patients receiving more than 20 U per day, and chlorpropamide was given in the same fashion

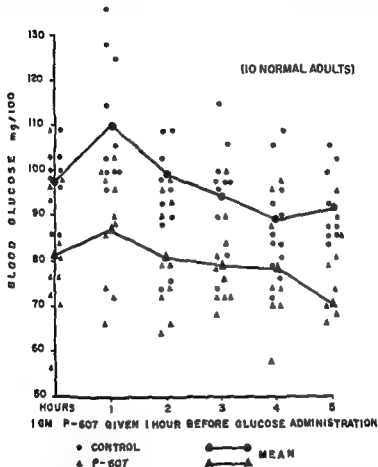


FIGURE 1. Changes in glucose tolerance test with chlorpropamide

described for the whole group. Further reductions in insulin were made according to the response.

Diabetic control was evaluated on the basis of glycosuria and glycemia. Urine sugar was checked three times a day by the qualitative Benedict's method, and the results were recorded in the usual way from 0 to + + + +. Blood glucose determinations were made by the Folin Wu method in the fasting state and, occasionally, after meals in the ambulatory cases.

TABLE 1  
AGE DISTRIBUTION OF PATIENTS

Age (years)	No. of cases
10-19	2
20-29	3
30-39	2
40-49	15
50-59	21
60-69	5
70-79	2

TABLE 2  
VASCULAR MANIFESTATIONS AND COMPLICATING DISEASES

Disorder	No. of cases
Retinopathy	27
Nephropathy	5
Cataract	7
Neuropathy	18
Urinary infection	3
Gangrene	1
Arteriosclerotic heart disease	11
Tuberculosis	2 (1 inactive)
None	14

TABLE 3  
DATA REGARDING PREVIOUS TREATMENT

Treatment	No. of cases
Diet alone	8
Insulin	33
Tolbutamide	4
Carbutamide	1
None	4

Outpatient cases were followed twice weekly and close clinical observation was maintained. Particular emphasis was placed on the detection of toxic manifestations. In twenty seven cases, liver function tests were done prior to the treatment and were repeated about three weeks later. These included the Bromsulphalein test and measurements of serum bilirubin, cephalin cholesterol flocculation, and thymol turbidity. Complete blood counts were done before chlorpropamide in forty cases and repeated toward the end

of the therapeutic trial. Because of the possibility of crytalin analysis was done in all cases.

### Results

According to the clinical and laboratory data every diabetic patient on tolbutamide treatment the diabetic patients were placed in three response groups (TABLE 4)

TABLE 4  
CRITERIA FOR CLASSIFICATION OF RESPONSE TO TOLBUTAMIDE

Classification	Characteristics
Good	At least three of the following a) Fasting blood sugar less than 130 mg per cent b) Postprandial blood sugar less than 170 mg per cent c) Zero to 1 plus glycosuria d) Complete or almost complete substitution (75 per cent reduction) in insulin requirements
Fair	a) Fasting blood sugar less than 150 mg per cent b) Postprandial blood sugar less than 200 mg per cent c) One plus to 2 plus glycosuria d) At least 50 per cent reduction in insulin requirements
Poor	All others

**Good response group** Constituting 56 per cent of the total patients in this group display at least 3 of the following characteristics: (1) a fasting blood sugar of less than 130 mg per cent, (2) a postprandial blood sugar less than 170 mg per cent, (3) zero to 1 plus glycosuria and (4) complete or almost complete substitution (75 per cent reduction) in insulin requirements.

**Fair response group** Patients (22 per cent of total) in this group show (1) a fasting blood sugar of less than 150 mg per cent, (2) postprandial blood sugar of less than 200 mg per cent, (3) one plus to 2 plus glycosuria and (4) at least 50 per cent reduction in insulin requirements.

**Poor response group** This group is constituted of all patients (22 per cent) not classified in the other groups.

Using the above criteria, which are related entirely to the control of the hyperglycemic and glycosuric aspects of diabetes, a good response was observed in 28 of 50 cases (56 per cent), a partial effect in 11 (22 per cent) and none in 11 (22 per cent).

### Discussion

an almost complete substitution (75 per cent) in 1

Two patients were on tolbutamide and 1 on carbutamide. There was a

transitory period of hyperglycemia and intense glycosuria immediately following the substitution of tolbutamide for chlorpropamide in 1 case, but glucose levels promptly returned to normal within the following 48 hours

The nine remaining patients were without insulin or other hypoglycemic agents at the start of the study. They failed to respond to diet alone, but responded to chlorpropamide

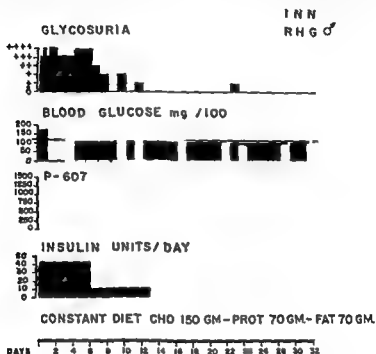


FIGURE 2

Fasting blood sugar values in the pretreatment period in the whole group ranged from 90 to 350 mg /100 cc, the average being 203 mg. After chlorpropamide administration, values varied from 79 to 161, the average being 110 mg /100 cc

The favorable response in the cases considered "good" occurred between the second and eighteenth days, the average at 5.5 days. In most patients, after controlling their diabetes, the average daily maintenance dose of chlorpropamide was 500 mg

An illustrative case is presented in FIGURE 2. The drug was well tolerated, but we noted slight hypoglycemia in three cases

Case 44 seemed to be a relative failure at the beginning, as there was a period of 17 days before glucose levels were controlled with 1 gm of chlorpropamide and decreasing doses of insulin. Ten days later we were able to discontinue insulin and to control the patient with chlorpropamide (FIGURE 3)

In the fair response group, 7 patients were receiving insulin in doses that ranged from 20 to 80 U per day, the average being 43 U—that is, 15 U more than the mean of the good response group. At the beginning of the study 1 patient was on tolbutamide, 2 were on diet alone (but their diabetes was uncontrolled) and 1 was receiving no treatment. Chlorpropamide administration was maintained for an average of 22 days, with a range of 8 to 38 days. The mean daily maintenance dose in this group was 770 mg.

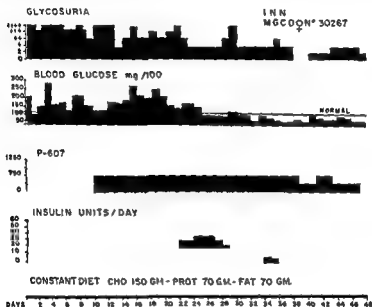


FIGURE 3

Average values for the fasting blood sugar dropped from 207 mg/100 cc to 152 mg/100 cc after chlorpropamide administration. A typical case is presented in FIGURE 3.

At the beginning of the study, 10 patients in the poor response group were receiving 25 to 60 U of insulin per day, the average being 37 U, and 1 was on tolbutamide but had not responded to this compound. Our five patients

propamide was given in a dose of 1 gm per day, the average duration of treatment was 12.6 days. A case is presented in FIGURE 5.

Also studied was the possibility of a correlation between the response to chlorpropamide and several factors such as the type of diabetes, the presence



of obesity, the previous insulin requirements, and the duration of the disease. The results are shown in FIGURE 6.

The only positive correlation found was that between response and disease type, as no single case of juvenile diabetes responded to chlorpropamide treatment.

*Side effects and toxic manifestations* Eleven patients complained of somnolence, weakness, and a "dizzy feeling" that were thought to be due to

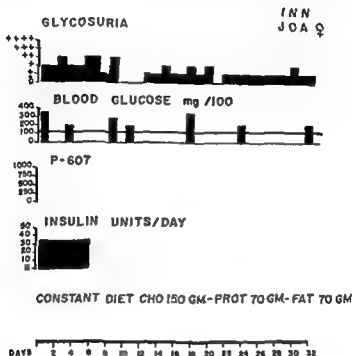


FIGURE 4

hypoglycemia, but glucose determinations failed to confirm this impression. Dermatological lesions were seen in 3, of these, 1 was an exfoliative dermatitis that promptly subsided after discontinuance of therapy. The other 2 patients had urticaria; this disappeared in 1 in spite of continuing treatment and, in the remaining case, after reducing the dose from 1 gm to 750 mg per day. Four patients complained of constipation.

No change in the liver function tests was shown in the 27 patients in whom such studies were done before and during chlorpropamide therapy.

Aside from transitory reduction in granulocytes in 3 cases, no toxic effects on the hematopoietic system were observed.

No crystalluria was found during the period of observation.

Thyroid function, as demonstrated by uptake of  $I^{131}$  by the thyroid gland, was measured in 7 cases before the onset of chlorpropamide treatment; this was repeated 2 to 3 weeks later, no significant depression was observed.

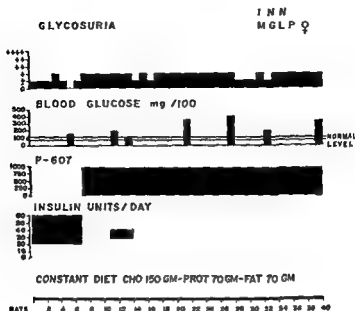


FIGURE 5

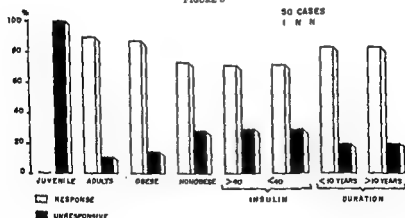


FIGURE 6 Response of diabetic patients to chlorpropamide

### Summary and Conclusions

Chlorpropamide was administered to ten normal healthy adults and fifty diabetic patients in order to study its hypoglycemic activity. A flattening of the glucose tolerance curve was seen after its administration.

Fifty six per cent of the diabetic patients responded favorably to the

was obtained

No alterations in liver function or in the hematopoietic system were observed

Side effects included somnolence weakness and dizziness in eleven cases

Response does not seem to be related to the duration of the diabetes to the insulin requirements (within certain limits) or to deviations from normal weight

## SUMMARY OF THE MONOGRAPH

Charles H. Best

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We all appreciate the fact that new evidence derived from the use of oral hypoglycemic agents has focused a searching light on the formation, liberation and action of insulin on a variety of metabolic processes and on diabetes and its treatment. We acknowledge again our debt to Loubatières<sup>1</sup> for his pioneering work and our indebtedness to our German colleagues<sup>2,4</sup> for their great and essential chemical and clinical contributions to this rapidly expanding field. I was just beginning to take a scientific interest in metabolism when the work on the hypoglycemic effect of guanidine was published in 1918.<sup>3</sup> We lived through the studies and clinical use of Synthalin,<sup>5</sup> Myrtilin and many other disappointing hypoglycemic agents.<sup>6</sup> However there is no doubt as we all realize that a new era of oral hypoglycemic therapy is here. There is every indication that if these drugs are carefully used a great deal of comfort and help can be given to many properly selected patients. It is fortunate that a large number of experienced people have contributed to this monograph in appraising the value of chlorpropamide.

There are a few papers that relate to insulin alone; this raises the total to fifty thousand plus a few since 1921. Robert Williams<sup>7</sup> has given us a comprehensive discussion of the destruction of insulin by homogenates and fractions of many tissues. The activity in tissues varies from the maximum in liver to the minimum in blood plasma. Williams makes the distinction between the reductive and proteolytic mechanisms of degradation (glucagon is destroyed only proteolytically). Little change in the insulin-destroying activity was noted after infective and traumatic insults. Traumatization of rat muscle *in vivo* did increase the insulin-destroying effect. Williams paid eloquent tribute to Mirsky's<sup>8</sup> original contributions in this field. In discussion Mirsky emphasized that in referring to insulinase he envisaged a labile saturated proteolytic system. Both Williams and his colleagues and Mirsky have now prepared a highly purified insulinase, and it is obvious that studies of the physiological role of this enzyme will be greatly facilitated.

Prout and Evans<sup>9</sup> have measured the *in vivo* disappearance of  $^{125}$ I insulin and feel that the fixation of the hormone by tissues is not a significant factor in this removal. The intact insulin is distributed in a volume similar to that of extracellular water; that is it is distributed throughout the extracellular space.

There are many problems on which we all seek further information. What is the mechanism of action of any of the oral hypoglycemic agents? What evidence is there that an increased secretion or liberation of insulin is produced? Loubatières provided our first evidence in a classically physiological way that is by removing the pancreas and noting the disappearance of the effect. Housay<sup>10</sup> and many other authors have confirmed this. Most of

<sup>1</sup> Eiser here in this monograph

the recent data have been obtained in studies on tolbutamide and the situation is still unsettled. I shall not attempt to review here all the direct and indirect evidence that has been obtained in a variety of ways. There have been studies on the morphological changes of the beta cells<sup>10-17</sup> and on the amounts of insulin extractable from the pancreas.<sup>18-19</sup> The direct attack—that is estimation of the insulin content of plasma—has many supporters.<sup>19-23</sup> Indirect approaches have utilized cross circulation tests (Loubatieres<sup>24</sup> Fda,<sup>27</sup> Cottrill<sup>28-29</sup>), perfusion experiments, eviscerated preparations,<sup>30-32</sup> and a great variety of metabolic studies—glucose tolerance tests<sup>33-37</sup> and estimations of such factors as serum inorganic phosphate,<sup>34-36, 39-40</sup> potassium,<sup>37-40</sup> nitrogen balance,<sup>41-43</sup> blood pyruvate<sup>36-37, 43-44</sup> and lactate,<sup>36-40, 41</sup> plasma UFA,<sup>45-48</sup> ketones,<sup>49-52</sup> and the RQ.<sup>53-55</sup> The effect on the decrease in sugar output by the liver<sup>54-57</sup> sometimes has been used, in effect as a test for insulin and changes in enzyme activity in the liver obviously might be similarly interpreted<sup>58</sup> in certain cases.

The importance of knowing the extent and duration of insulin liberation produced by the various oral hypoglycemic agents cannot be overemphasized. How can we know to what extent insulin itself changes enzyme activity in the liver and how much direct action a drug is exerting without this information? The valuable studies of Sass and Handelsman\* on transaminases in liver homogenates of animals that have received chlorpropamide illustrate this point. Indirect approaches can help of course. The situation is similar when we consider sugar production by the liver, as I shall point out later.

I shall not attempt to give an appraisal of all the indirect evidence that is very strongly in favor of some insulin liberation after administration of the oral agents. Positive direct evidence has been reported by von Holt,<sup>19</sup> Goetz,<sup>20</sup> and Pfeiffer *et al*.<sup>25</sup> The methods used are not above criticism by the investigators themselves as well as by others. Recently, Renold (personal communication) and his group were unable even with their very sensitive and reliable method, to detect in man any increase in plasma insulinlike substances after administration of the oral agents. They do find an increase in the amount of these materials in pancreatic vein blood of the dog. A survey of this field leads to the conclusion that one aspect of the action of the hypoglycemic oral agents is almost certainly the liberation of a certain amount of extra insulin, but the possibility remains that the mere presence of a little insulin furnishes the essential link.

ported this conclusion. In human subjects he found that a slow infusion of insulin produced a decreased hepatic output of glucose, but no indication of increased peripheral use. Larger doses of insulin gave both effects.

Madison, Unger, and Kaplan\* found in human subjects that slow infusion of glucagon-free insulin, which produced a good fall in arterial blood sugar, caused no change in arteriovenous difference. Tolbutamide produced the same effect. They reported that in dogs with portacaval fistulas the administration of insulin consistently produced a decreased hepatic output of sugar. These workers emphasized the various differences between endogenous and exogenous insulin. The only established difference between natural and exogenous insulin relates to the route of entry of the hormone. We all know that depancreatized dogs and some severe diabetic patients have lived a life time (that is, six years and thirty seven years, respectively) on exogenous insulin.

Schambye and Tarding\* found that tolbutamide produced an inhibition of hepatic glucose output as judged by plateauing of the curve of specific activity of  $C^{14}$  glucose in normal and depancreatized dogs and in diabetic patients. The inhibition in the normal organism lasted no longer than 40 min. but in the diabetic it lasted at least 2 hours. Although insulin given intravenously and intraportally in normal dogs could not be found to decrease hepatic output of sugar, tolbutamide did produce this effect. These differences in the observed effects of insulin emphasized again the necessity of attempting to standardize our tools—the type and amount of insulin, for example and many other variables.

I propose now to emphasize a point or two on the effect of insulin on the liver. Perfusion of the isolated liver (Miller, Hamff\*) has demonstrated a rather prompt effect of insulin on fat formation, and I shall not be surprised to see revealed a prompt, direct action on sugar and amino acids in this tissue. The fundamental interest and practical importance of this subject must be obvious. We cannot readily investigate the direct action of the oral agents on liver if these agents liberate insulin which, in turn, affects the liver. There is, of course, no doubt about many very important delayed and perhaps indirect actions of insulin on the liver. De Dure<sup>51</sup> and his colleagues recently discussed this matter at the International Diabetes Federation in Dusseldorf, Germany. While I feel that small amounts of insulin are probably liberated or, at least, are necessary for the action of the oral agents, I must stress the fact that the exploration of the direct effect of these agents on the liver has hardly been started. Downie\* has aroused our interest by reference to Bornstein's<sup>52</sup> work on the effect of chlorpropamide on the oxidation of  $C^{14}$ -labeled alanine to  $CO_2$  in liver slices. Mirsky\* has stimulated us again with his finding that tolbutamide prevents the diabetogenic action of somatotropin in dogs. Zarowitz\* compared the glucose and tolbutamide tolerance tests in nondiabetic and diabetic patients. He feels that the tolbutamide test detects some mild diabetics that the older test would not reveal. Tolstoi\* did not see the advantage of and justification for the tolbutamide test.

However, we are concerned particularly in this monograph with chlor-

\* Elsewhere in this monograph.

propamide. It is obvious that this compound is an effective agent and that, under certain circumstances, it can be given safely for prolonged periods to humans.

McLamore's work\* gives us a great deal of information on the relation of chemical structure to hypoglycemic action. Schneider† and Mary Root,‡ agreed on the greater activity of chlorpropamide as compared to tolbutamide in mice, rats, and dogs. The former was slightly more toxic. Chlorpropamide was active in monkey.

In rats there was a degranulation of beta cells, no effect in alloxan diabetes, a positive effect in hepatectomized preparations and an enhanced effect in adrenalectomized animals. Schneider reported certain toxic effects (predominately on the CNS) with large doses of chlorpropamide in dogs, but he stated that 120 mg/kg was well tolerated for 20 months. There was no change in various liver function tests, this is in contrast to the findings of A. Sirek and O. Sirek<sup>22</sup> in our laboratory with tolbutamide which, at a level of 30 mg/kg, does produce hepatic injury in normal and diabetic dogs. We should test chlorpropamide under the same conditions, using dosages that produce the same hypoglycemic effects. The liver lesions seen in a few patients receiving chlorpropamide resemble strikingly those seen in dogs given tolbutamide, but the finding in dogs must not be "transferred" to human subjects, the striking absence of liver lesions in many, many thousands of patients receiving tolbutamide must be emphasized again.

Lundbaeck\* and Clarke\* differ in their finding of glucose uptake by diaphragm in *in vitro* studies. Lundbaeck has reported an increased carbohydrate tolerance in patients given chlorpropamide or tolbutamide. Danowski\* has studied chlorpropamide in carefully standardized normal and diabetic subjects. He feels that the first effect may be to decrease gluconeogenesis, the second, perhaps, to increase insulin liberation. He studied particularly blood glucose and phosphate detecting no change in serum inorganic phosphate in diabetics given chlorpropamide. His report on the increased immediate utilization of sugar and the fall in serum phosphate after cortisone is intensely stimulating.

On the clinical use of chlorpropamide, all agree, as Tolstoi summarized, that chlorpropamide is an effective agent, and one more potent than tolbutamide. As Beaser emphasized, the drug should be criticized only for its

effectiveness at a normal effective dose.

blood level and  
amide the same

effects were produced. Craig\* agreed but others have been less certain of the significance of this relationship. Kelly West\* feels that chlorpropamide is more potent intrinsically, but he has not as yet measured blood levels. Beaser, using different doses of chlorpropamide, found a direct relationship between dose and blood level, but not necessarily between dose and blood sugar values. Hamff\* reported three cases of jaundice with the use of large

\* Elsewhere in this monograph

† Of Chas. Pfizer & Co. Inc. Brooklyn, N. Y.

‡ Of Eli Lilly and Company, Indianapolis, Ind.

doses of chlorpropamide. Two of these patients later did well on smaller doses and the third was transferred to tolbutamide.

There have been primary and secondary failures with both drugs and, in certain cases, the other drug was effective. Nabarro's\* statement on the incidence of acquired resistance to tolbutamide was noteworthy, as many as 14 per cent of his cases showing resistance in 18 months. Insulin consistently has been found to be effective, and its dose can safely be increased when necessary.

The selection of cases for oral therapy is a vitally important matter. Duncan,\* Hamwi,\* and many others have discussed this problem. Ketosis on withdrawal of insulin is considered to contraindicate the use of the oral agents. In addition to careful selection of patients, Duncan emphasized that these drugs should control diabetes at least as well as insulin in mild cases and, if used with insulin in the more severe ones, dramatic evidence of their effectiveness should be available.

I was very interested in Stowers' \* paper on competition for tubular excretion between chlorpropamide and salicylates. Salicylates raised the level of chlorpropamide in plasma, this finding may have a bearing on Segarra's\* work in tuberculous patients who were receiving both drugs.

Henry Ricketts\* stressed the necessity for a uniform method of clinical investigation when drugs are to be compared. All the established principles of good treatment of diabetes must be followed before a new variable is introduced.

The precise incidence of the complications produced by chlorpropamide in therapeutic doses is not known. It must be emphasized that each complication should be most thoroughly investigated. The evaluation of the significance of complications in the older age group is, of course, particularly difficult.

### *Concluding Remarks*

I have approached this subject as a physiologist and not as a clinician. We have in chlorpropamide an effective agent that appears to be more potent than the two drugs previously used. Adequate dose response curves are not yet available. Chlorpropamide is longer acting than tolbutamide and can be given in smaller doses, which should always be small and should never exceed 750 mg per day. It is probable that 500 mg or less would be adequate in most patients, and this amount would be safer. The safe therapeutic range of chlorpropamide is not yet determined. To this point, the safe therapeutic range of chlorpropamide has not yet been determined.

high levels and small increases in dose may have a surprisingly great effect on blood levels. Chlorpropamide tends to accumulate, and hypoglycemia may be disturbing, particularly during the early period of adjustment. Patients should be watched very carefully, since delayed responses inde-

\* Elsewhere in this monograph



and is illustrated again by a case presented by Tolstoi. Wherever possible patients should be standardized on diet alone. If more specific therapy is needed the insulin requirement of all patients should be determined. If an oral agent is then used its insulin equivalent is thus made available. These are valuable data and they should be obtained whenever possible.

Someone has observed that the patients who respond best to the oral agents are those who do not need them. There is, of course, a real need for these agents in the right cases but it is becoming increasingly obvious that many patients are receiving these drugs who could and should be controlled by diet alone. As Dobson said the oral agents are frequently a convenience and not a necessity. In ten years we shall know if this convenience will have been perfectly safe.

I have attempted to quote a number of the distinguished clinicians and I conclude by referring to the finding made by Alexander Marble\* that chlorpropamide is an effective agent. Marble encountered a disturbing side effect in one patient of seventy two even with a relatively small dose of the drug. He believes as I do that there are many advantages in having a number of safe active oral agents available and he stressed the fact that more exact clinical work on chlorpropamide is needed to determine its proper sphere of usefulness.

It is important that the national diabetes associations intensify their efforts at the present time to help physicians in the selection of cases and in development of an appreciation of the risks involved and the benefits to be obtained from the oral hypoglycemic agents.

#### Acknowledgments

I am indebted to my Toronto colleagues A. Sirek and O. Sirek, Donald Clarke and Bernard Leibel for providing me with information in the preparation of this paper.

#### References

1. LOUBATIERES, A. 1946. Physiologie et pharmacodynamie de certains dérivés sulfamidés hypoglycémisants. Contribution à l'étude des substances synthétiques à tropisme endocrinien. Thèse Doct. Sci. naturelles Montpellier No 86. Causse Graille et Castelnau. Eds. Montpellier France.
2. FRANK, H. & J. FUCHS. 1955. Ein neues antidiabetisches Prinzip. Ergebnisse klinischer Untersuchungen. Deut. med. Wochschr. 80: 1449.
3. SCHULZ, J. D. & H. HARDEBECK. 1955. Über eine neue blutzuckersenkende Substanz. Deut. med. Wochschr. 80: 1452.
4. BERTRAM, F., E. BENDFELD & H. OTTO. 1955. Über ein wirksames perorales Antidiabeticum. Deut. med. Wochschr. 80: 1455.
5. WATANABE, C. K. 1948. Studies on the metabolic changes induced by administration of guanidine bases. J. Biol. Chem. 33: 253.
6. FRANK, E., M. NOTHMANN & A. WAGNER. 1926. Über synthetisch dargestellte Körper mit insulinartiger Wirkung auf den normalen und diabetischen Organismus. Klin. Wochschr. 5: 2100.
7. ALLEN, P. M. 1927. Blueberry leaf extract. J. Am. Med. Assoc. 89: 1577.
8. MAIER, WEINERTSGRUB, D. 1937. Guanidinderivate beim Diabetes Mellitus. Deut. med. Wochschr. 63: 1400.

\* Elsewhere in this monograph.

- 9 HOUSSAY, B A, J C PEYROS N TEODOSIO J BOWLETT & J APFELBAUM 1957 Action of the hypoglycemic sulfonyl compounds in hypophysectomized adrenalectomized and depancreatized animals *Ann N Y Acad Sci* 71(1) 12
- 10 CREUTZFELDT W & H FINTNER 1936 Blutzucker und histologische Veränderungen nach D860 bei normalen Ratten *Deut med Wochschr* 81 892
- 11 ASHWORTH M A & R E HAIST 1956 Some effects of BZ 55 (carbutamide) on the growth of the islets of Langerhans *Can Med Assoc J* 74 975
- 12 FREIFFER, F F H STEIGERWALD W SANDRITTER A BANDER A MAGER U BECKER & K. RATTEN 1957 Vergleichende Untersuchungen von Morphologie und Hormongehalt des Langerhansschen Inseln nach Sulfonylharnstoffen (D860) *Deut med Wochschr* 82 1568
- 13 CREUTZFELDT W L DETTING & O WEITE 1957 Das B Zellsystem von normalen und hypophysectomierten Ratten sowie Kaninchen unter D860 und diabetogenen Hormonen *Deut med Wochschr* 82 1564
- 14 BANDER A A HAYESLEFR & J SCHOLT 1957 Ergänzende pharmakologische Untersuchungen über Rastidon *Deut med Wochschr* 82 1557
- 15 GEPTS W 1958 Die histopathologischen Veränderungen der Langerhansschen Inseln und ihre Bedeutung in der Frage der Pathogenese des menschlichen Diabetes *Endokrinol* 36 185
- 16 KRACHT, J 1958 Experimentelle Morphologie des Inselorgans unter BZ55 D860 und IPTD 40 (Abstracts) 3rd Congr Intern Diabetes Federation Dusseldorf Germany
- 17 VOLK B W M G GOLDFYER S WEINERFELD & S S IANARUS 1957 Functional and histological studies concerning the action of sulfonylureas *Ann N Y Acad Sci* 71(1) 141
- 18 ROOT M A 1957 Effect of carbutamide on the insulin content of the dog pancreas *Diabetes* 6 12
- 19 VON HOLT C L VON HOLT J KRACHT D KRONER & J KUBENAU 1957 Carbutamide and plasma insulin like activity *Science* 126 735
- 20 KRACHT J B KRONER L VON HOLT & C VON HOLT 1957 Zunahme von Plasma insulinaktivität und B Zellenmitosen nach N (4 Methyl benzolsulfonyl) N Butyl harnstoff *Naturwiss* 44 16
- 21 RENOLD A E D B MARTIN B R BOWELL & G W THORN 1957 Studies on the site of the aryl sulfonylureas in man II *Ann N Y Acad Sci* 71(1) 71
- 22 GOETZ F C & R H ECKHAHL 1958 Direct demonstration of insulin release from pancreas by tolbutamide *Federation Proc* 17 55
- 23 WEAVER J A T E PROOT G W SCOTT & S P ASPER 1958 Role of insulin in acute hypoglycemic action of tolbutamide *Brit Med J* 5068 425
- 24 CONRAD V 1958 Etude du mécanisme d'action des sulfamides hypoglycémisants 100 Abstr 3rd Congr Intern Diabetes Federation Dusseldorf Germany
- 25 FREIFFER L P A E RENOLD D B MARTIN A DAGENAIS J W MERRIN D H NELSON G SHOREMAKER & G W THORN 1958 Untersuchungen über die Rolle des Pankreas im Wirkungsmechanismus blutzucker senkender Sulfonylharnstoffe 44 Abstr 3rd Congr Intern Diabetes Federation Dusseldorf Germany
- 26 LOUBATIERES A 1955 L'utilisation de certaines substances sulfamidées dans le traitement du diabète sucré expérimental *La Presse Med* 63 1701 1703
- 27 POZZA G G GALANSINO & P P FÖR 1956 Insulin secretion following carbutamide injections in normal dogs *Proc Soc Exptl Biol Med* 82 539-542
- 28 COLWELL A R JR J A COLWELL & R COLWELL SR 1956 Intrapaneatic perfusion of the antidiabetic sulfonylureas *Metabolism* 5 749
- 29 COLWELL, A R JR J A COLWELL & R COLWELL SR 1957 Perfusion studies with sulfonylureas in dogs *Ann N Y Acad Sci* 71(1) 125
- 30 FRITZ I B J A MORTON M WEINSTEIN & R LEVINE 1956 Studies on the mechanism of action of the sulfonylureas *Metabolism* 5 744
- 31 HOUSSAY B A J C PEYROS E URGOTY N TEODOSIO J APFELBAUM & J BOWLETT 1957 The role of insulin in the action of the hypoglycemic sulfonyl compounds *Ann N Y Acad Sci* 71(1) 25

- 32 DELIV W E & R L JOHNSON 1957 Studies concerning the role of the liver in the hypoglycemic response of animals to tolbutamide *Ann N Y Acad Sci* 71(1) 177
- 33 HEINEMANN A C CORN M WEINSTEIN & R LEVINE 1956 Clinical experience with carbutamide and tolbutamide *Metabolism* 5 972
- 34 PLURNELL R V WAI E PRATT C HLAD JR & H ELERICK 1956 Some observations on the mode of action of Orinase *Metabolism* 5 778
- 35 VOLK B W S WEISENFELD S S LAZARUS & M G GOLDBER 1956 Mechanisms of action of the hypoglycemia producing sulfonylurea derivatives *Metabolism* 5 894
- 36 RENOLD A F A I WINEGRAD F R FROESCH & G W THORV 1956 Studies on the site of action of the arylsulfonylureas in man *Metabolism* 5 757
- 37 FAJAN S S L H LORIS A R HENNES B L WAJCHENBERG R D JOHNSON R D GITTLES I P ACKERMAN & J W CONN 1957 Metabolic effects of sulfonylureas in normal men and in various types of diabetic patients *Ann N Y Acad Sci* 71(1) 207
- 38 GOETZ F C A S GILBERTSEY & A JOSEPHSON 1956 Acute effects of Orinase on peripheral glucose utilization *Metabolism* 5 788
- 39 MOUNIKÉ G & H BIRGEREL 1956 Die Wirkung von BZ55 und D860 auf den Blutzucker und den Serumspiegel an Phosphor Kalium und Natrium von Hunden *Deut med Wochenschr* 81 900
- 40 SZUCS S & A TISZAI 1958 Effects of carbutamide on blood levels of glucose potassium and inorganic phosphate *Diabetes* 7 288
- 41 GOETZ F C 1957 Respiratory quotient and nitrogen balance during tolbutamide administration *Ann N Y Acad Sci* 71(1) 46
- 42 SFEIGERWALD H & SCHOFFLING & C F PFLEITER 1956 Das Verhalten von Aceton Acetessigsäure  $\beta$ -Oxybuttersäure  $\alpha$ -Ketoglutaräure Brenztraubensäure und Milchsäure nach D860 *Deut med Wochenschr* 81 837
- 43 MILLER M J W CRAIG M S MACKENZIE W R DRUCKER M CAMMARN L H WOODWARD 1957 Studies of the effect of intravenous tolbutamide on pyruvic and lactic acid concentrations in peripheral venous blood in normal and diabetic subjects and on splanchnic metabolism of fructose and glucose *Ann N Y Acad Sci* 71(1) 51
- 44 MOORHOUSE J A & R M KARR 1956 Physiologic actions of Orinase and their relationship to the types of diabetes in man *Metabolism* 5 847
- 45 BIERMAN E L T A ROBERTS & A P DOLE 1957 Effect of tolbutamide (Orinase) on plasma non-esterified fatty acids *Proc Soc Exptl Biol Med* 95 437
- 46 STOTTER G I SEIDLER T DORNHILLER M FURTHMILLER & W ENDRES 1957 Report on experiences in one and a half years of oral treatment of diabetes with tolbutamide *Ann N Y Acad Sci* 71(1) 280
- 47 ANDERSON G L A J PERFETTO C M TERMINE & R R MONACO 1956 Hypoglycemic action of Orinase Effect on output of glucose by liver *Proc Soc Exptl Biol Med* 92 340
- 48 ASHMOKE J G F CAHILL A S EARLE & S ZOTTE 1958 Studies on the disposition of blood glucose A comparison of insulin and Orinase *Diabetes* 7 1
- 49 MANNNER T P SCHAMBEY & F TARDING 1958 Studies of the glucose exchange in the normal and diabetic man by liver catheterization and  $C^{14}$  glucose 35 *Abstr 3rd Congr Intern Diabetes Federation Dusseldorf Germany*
- 50 WEBER G & A CANTERO 1958 Effect of Orinase on hepatic enzymes involved in glucose-6-phosphate utilization *Metabolism* 7 333
- 51 DE DUVE C 1958 Insulin and liver metabolism 5 *Abstr 3rd Congr Intern Diabetes Federation Dusseldorf Germany*
- 52 BORNSTEIN J 1957 Inhibition of alanine transaminase by the hypoglycemic sulfonylurea derivatives *Nature* 179 534
- 53 SIREK A O SIREK F C MOORHOUSE J LOGOTHETOPOLLOS & C H BEST 1958 Effects of prolonged administration of tolbutamide to normal and depancreaized dogs 62 *Abstr 3rd Congr Intern Diabetes Federation Dusseldorf Germany*

